# Carotenoids and Related Compounds

## GENERAL REMARKS AND ANALYTICAL PROCEDURES

The first step in the preparation of many pure carotenoids is extraction from a natural source. This extraction is made as gently as possible, to prevent the isomerization and destruction of the carotenoids. The preferred method uses extraction of the carotenoids with a chilled solvent. One quite satisfactory method involves the homogenization of plant material in a mixture of acetone and petroleum ether in a Waring Blendor. Frequently, solid carbon dioxide is added to keep the solution cold. Antioxidants and basic compounds (e.g., calcium carbonate) may also be added, to minimize oxidation of the carotenoids or their destruction by plant acids. Other methods involve grinding with solvent in a mortar or extracting with boiling solvent (methanol or ethanol, for example). The carotenoids are transferred to a hydrocarbon solvent (petroleum ether or benzene) after the addition of water and salt (sodium chloride or ammonium sulfate) to the alcohol or acetone phase. Xanthophylls are then separated from the carotenes by partition between immiscible solvents. Most commonly, this separation is effected with petroleum ether and 90% methanol. However, other solvent mixtures are also used. The xanthophylls are found in the methanol (hypophase), and the carotenes are present in the petroleum ether (epiphase). If xanthophyll esters are present, the carotene solution is saponified with alcoholic potassium hydroxide (preferably at room temperature or below), and the resultant xanthophylls are separated from the carotenes by partition between petroleum ether and aqueous alcohol. Following separation and thorough removal of water from the hydrocarbon solvent, the carotenes are separated from one another by chromatography. The xanthophylls are also transferred to petroleum ether, the solution is dried, and the pigments are separated by chromatography. The separated compounds (carotenes or xanthophylls) may then be crystallized or stored in a nonpolar solvent at −10 to −20 °C. Similar procedures are used for the purification of chemically synthesized carotenoids. To minimize *cis–trans* isomerization of the carotenoids, all operations should be carried out in dim light or semidarkness and as rapidly as possible. Pure solvents should be used, to minimize destruction of the carotenes, and, when possible, operations should be conducted in an inert atmosphere.

The most useful criteria of purity for carotenoids are chromatography and absorption spectra. Colored impurities in a carotenoid preparation are readily detected by chromatography. Thin-layer, paper, or column chromatography may be used, with preference to the first two because they are more rapid and require a smaller amount of material. Colored impurities are also detected by examination of the absorption spectrum. The presence of *cis*-isomers in a carotenoid preparation may be determined by chromatography or by examination of the absorption spectrum. Most *cis*-isomers have pronounced "*cis*-peak" absorption. Colorless impurities in a carotenoid preparation are detected by a comparison of the absorption coefficients of the carotenoid with those of the preparation.

Several other methods are quite useful for establishing the presence or absence of impurities in a carotenoid preparation. Infrared spectra will show the presence of colored or colorless impurities. However, this

method may not reveal the presence of small proportions of impurities. Nuclear magnetic resonance and mass spectrometry are more useful in establishing the purity of a carotenoid preparation. Gas–liquid chromatography of hydrogenated carotenoids has also been used to establish purity; this method is particularly useful if the column is programmed from about 150 to 280 °C.

Carotenoids are decomposed by light, air, and acid solvents. Therefore, it is recommended that they be stored in the dark (preferably in brown vials), in an inert atmosphere (or a sealed ampoule), at −10 to −20 °C.

The colorless compounds related to the carotenes, or to precursors in their biosynthesis, are assayed for purity by a variety of methods. These methods are detailed in the specifications for each compound.

The presentation of physical characteristics of the carotenoids and related compounds is uniform throughout these specifications. Thus, all light-absorption wavelength maxima are given in nanometers (nm), and all melting points and boiling points are given in degrees Celsius (°C).

Melting points are reported as stated in the particular reference cited. Since the melting point of a carotenoid can vary appreciably, depending upon the method used for its determination, original articles should be consulted when a comparison is made between an experimentally determined melting point and a value reported in these specifications.

The references listed for a compound reported in these specifications should be consulted for the synthesis, isolation from natural materials, purification, and assay for purity of that compound. In addition, the following bibliography may be consulted for a wide range of information of value in the preparation of carotenoids and in the analysis of these compounds for purity.

Following the bibliography are the names of contributors and reviewers who particularly assisted the Subcommittee and whose work is hereby gratefully acknowledged.

## ANALYSES OF COMMERCIAL PRODUCTS

Analyses were carried out, during 1969, to determine the purity of 16 commercially available carotenoids and related compounds. The compounds assayed were purchased on the open market, and they were ones for which criteria and specifications had been reported in Publication No. 1344 of the National Academy of Sciences, the previous edition of the present publication. These compounds varied considerably in purity. Seven carotenoids and related compounds had purities of 90–100% by the criteria reported in the earlier publication, and seven had purities of 80–90%. One compound had a purity of 70–80%, and one had a purity of less than 10%. None of the commercial products contained colored impurities. Hence, the impurities were either colorless compounds that had not been removed in the preparation of the commercial product or colorless oxidation products that were formed during the time interval between their preparation by the commercial supplier and our receipt of the product. It seems probable that the product of very low purity was one in which much oxidation occurred after its preparation by the supplier. Such a product would, however, be almost worthless to the purchaser.

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S. P. Colowick and N. O. Kaplan, *Methods in Enzymology: Steroids and Terpenoids*, Vol. XV, R. B. Clayton, ed., Academic Press, New York (1969).

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J. W. Porter and D. G. Anderson, in *Chromatography*, E. Heftmann, ed., Reinhold Publishing Corp., New York (1961), p. 465.

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B. C. L. Weedon, *Pure Appl. Chem.*, 20, 531 (1969).

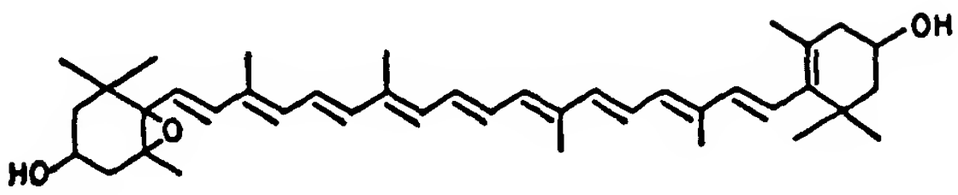
L. Zechmeister, cis–trans-*Isomeric Carotenoids, Vitamins A and Arylpolyenes*, Springer-Verlag, Vienna (1962).


## CONTRIBUTORS AND REVIEWERS

A. G. Andrewes, The Norwegian Institute of Technology, Trondheim, Norway
G. Britton, The University of Liverpool, Liverpool, England
Peter H. W. Butterworth, University College, London, England
C. J. Chesterton, Royal Postgraduate Medical School, London, England
B. H. Davies, University College, Aberystwyth, Wales
B. C. L. Weedon, Queen Mary College, London, England

## Carot-1
## Antheraxanthin
## 5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3′-diol

**Formula:** $C_{40}H_{56}O_3$
**Formula Wt.:** 584.89
**Calc. %:** C, 82.14; H, 9.65; O, 8.21




Antheraxanthin has been characterized as 5,6-epoxy-3,3′-dihydroxy-β-carotene.[1,2] The stereochemistry of this compound is unknown.

**Sources:**

*Natural Sources.* Antheraxanthin is present as an ester in *Lilium tigrinum*,[3] from which it was originally isolated. It is also present in smaller proportions in some fruits.

*Partial Synthesis.* Antheraxanthin is obtained on oxidation of zeaxanthin with monoperphthalic acid.[1,2,4] Two isomers, having different optical rotatory dispersion properties, are obtained.[4]

**Isolation Procedures:**[1-3] Antheraxanthin is extracted from biological materials with acetone or ethanol and then transferred to benzene. This pigment is then separated from other carotenoids by chromatography on a calcium hydroxide–Celite column.

**Methods of Purification:**

*Solvent Partition.* A partial purification of antheraxanthin can be obtained by partition between several solvent combinations.[6]

*Chromatography.* Antheraxanthin has been purified by chromatography on columns of calcium hydroxide[3] and of zinc carbonate.[1]

*Crystallization.* Needles or thin plates are obtained on crystallization from benzene–methanol.[3]

*Derivatives:* No derivatives other than the furanoid rearrangement product (mutatoxanthin) have been reported.[3]

**Methods of Assaying for Purity:**

*Chromatography.* Assays for chromatographic homogeneity can be carried out on thin-layer plates[6] or on kieselguhr paper.[7]

*Visible Spectrum.* Carbon disulfide:[3] 478 and 510 nm. Chloroform:[3] 460.5 and 490.5 nm.

*Mass Spectrum.* The mass spectrum of antheraxanthin has been reported.[8]

*Melting Point.* Antheraxanthin melts[3] at 205 °C.

*Derivatives.* Mutatoxanthin is obtained on treatment of antheraxanthin with chloroform containing a trace of hydrochloric acid.[1]

**Probable Impurities:** Violaxanthin and mutatoxanthin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**

1. P. Karrer and E. Jucker, *Helv. Chim. Acta*, 28, 300 (1945).
2. P. Karrer and E. Jucker, *Carotenoids*, E. A. Braude (translator), Elsevier, New York (1950).
3. P. Karrer and A. Oswald, *Helv. Chim. Acta*, 18, 1303 (1935).
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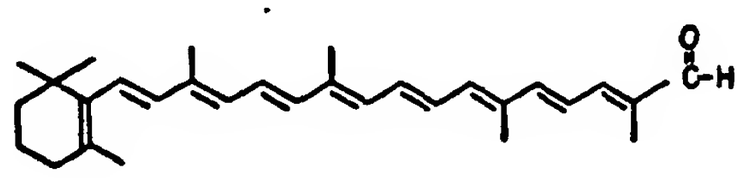
6. H. R. Bolliger, A. König, and U. Schwieter, *Chimia*, 18, 136 (1964).
7. A. Jensen and S. Liaaen-Jensen, *Acta Chem. Scand.*, 13, 1863 (1959).
8. H. Budzikiewicz, H. Brzezinka, and B. Johannes, *Monatsh. Chem.*, 101, 579 (1970).



## Carot-2
## β-Apocarotenal
## 8′-Apo-β-caroten-8′-al
## (β-Apo-8′-carotenal)

**Formula:** $C_{30}H_{40}O$
**Formula Wt.:** 416.65
**Calc. %:** C, 86.48; H, 9.68; O, 3.84




**Sources:**

*Natural Sources.* β-Apocarotenal has been reported to be present in citrus fruits,[1,2] various vegetables,[1] grass, [1,3] liver,[1] and duodenal mucosa.[4]

*Chemical Synthesis.* β-Apocarotenal has been synthesized from "β-$C_{15}$-aldehyde."[5] It has also been prepared by oxidation of β-carotene with potassium permanganate.[6,7]

**Methods of Purification:** β-Apocarotenal is purified by recrystallization from organic solvents (petroleum ether or petroleum ether–ethyl acetate).

**Methods of Assaying for Purity:**

*Chromatography.* β-Apocarotenal may be assayed for purity by chromatography on a column of partially deactivated alumina. It may also be chromatographed on thin-layer plates of silica gel G (Merck) or secondary magnesium phosphate. Cyclohexane–ethyl ether (8:2), carbon tetrachloride, or petroleum ether–ethyl ether (19:1) may be used as the developing agent.

*Visible Spectrum.* Cyclohexane: $E^{1\%}_{1cm}$ 2640 at 461 nm; 2165 at 488 nm.

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum has been reported.[8]

*Melting Point.* 139 °C, violet plates from methanol.[9]

**Probable Impurities:** *cis*-Isomers of β-apocarotenal.

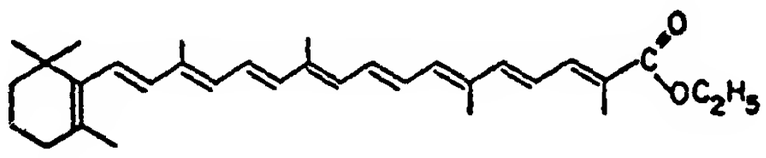
**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

**References**

1. A. Winterstein, A. Studer, and R. Rüegg, *Chem. Ber.*, 93, 2951 (1960).
2. H. Thommen, *Naturwissenschaften*, 49, 517 (1962).
3. H. Thommen and O. Wiss, *Z. Ernaehrungswiss. Suppl.*, 3, 18 (1963).
4. A. Winterstein, *Angew. Chem.*, 72, 902 (1960).
5. R. Rüegg, M. Montavon, G. Ryser, G. Saucy, U. Schwieter, and O. Isler, *Helv. Chim. Acta*, 42, 854 (1959).
6. P. Karrer and U. Solmssen, *Helv. Chim. Acta*, 20, 682 (1937).
7. P. Karrer, U. Solmssen, and W. Gugelmann, *Helv. Chim. Acta*, 20, 1020 (1937).
8. U. Schwieter, G. Englert, N. Rigassi, and W. Vetter, *Pure Appl. Chem.*, 20, 365 (1969).
9. P. Karrer and E. Jucker, *Carotenoids*, E. A. Braude (translator), Elsevier, New York (1950).

## Carot-3
## β-Apocarotenoic Acid Ethyl Ester
## Ethyl 8′-Apo-β-caroten-8′-oate
## [β-Apo-8′-carotenoic Acid ($C_{30}$) Ethyl Ester]

**Formula:** $C_{32}H_{44}O_2$
**Formula Wt.:** 460.71
**Calc. %:** C, 83.42; H, 9.62; O, 6.96



**Sources:**

*Natural Sources.* β-Apocarotenoic acid is a metabolic product of β-apocarotenal.[1,2] β-Apocarotenoic acid has been isolated from maize.[3]

*Chemical Synthesis.* Ethyl β-apocarotenoate is prepared in analogy to the methyl ester[4] from 15,15′-dehydro-10′-apo-β-carotenal ($C_{27}$) and (1-ethoxycarbonylethyl)triphenylphosphonium bromide.

**Methods of Purification:** Ethyl β-apocarotenoate is purified by crystallization from organic solvents (petroleum ether or petroleum ether–ethyl acetate).

**Methods of Assaying for Purity:**

*Chromatography.* Ethyl β-apocarotenoate is assayed for purity by chromatography on a column of partially deactivated alumina or on thin layers of secondary magnesium phosphate or alkaline silica gel G (Merck). Petroleum ether–ethyl ether (19:1) is used to develop the thin-layer chromatogram.

*Visible Spectrum.* Cyclohexane: $E^{1\%}_{1\,cm}$ values are 2550 at 449 nm and 2140 at 475 nm. The absorption spectra of β-apocarotenoic acid ethyl ester in hexane, ethanol, and petroleum ether have been published.

*Melting Point.* A range of 134–138 °C has been reported.

**Probable Impurities:** *cis*-Isomers of β-apocarotenoic acid ethyl ester.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**


1. O. Wiss and H. Thommen, *Carotine und Carotinoide*, K. Lang, ed., D. Steinkopff Verlag, Darmstadt (1963), p. 179.
2. H. Thommen, *Chimia*, 15, 433 (1961).
3. J. Baraud, F. Benitez, L. Genevois, and A. Maurice, *Compt. Rend.*, 260, 7045 (1965).
4. O. Isler, W. Guex, R. Rüegg, G. Ryser, G. Saucy, U. Schwieter, M. Walter, and A. Winterstein, *Helv. Chim. Acta*, 42, 864 (1959).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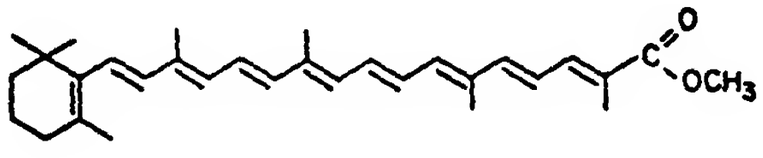
## Carot-4
## β-Apocarotenoic Acid Methyl Ester
## Methyl 8′-Apo-β-caroten-8′-oate
## [β-Apo-8′-carotenoic Acid ($C_{30}$) Methyl Ester]

**Formula:** $C_{31}H_{42}O_2$
**Formula Wt.:** 446.68
**Calc. %:** C, 83.36; H, 9.48; O, 7.16



**Sources:**

*Natural Sources.* β-Apocarotenoic acid is a metabolic product of β-apocarotenal.[1,2]

*Chemical Synthesis.* The methyl ester of β-apo-8′-carotenoic acid ($C_{30}$) is prepared from 15,15′-dehydro-β-apo-10′-car tenal ($C_{27}$) and (1-methoxycarbonylethyl)triphenylphosphonium bromide.[3]

**Methods of Purification:** The methyl ester of β-apocarotenoic acid is purified by recrystallization from petroleum ether or petroleum ether–ethyl acetate.

**Methods of Assaying for Purity:**

*Chromatography.*[1–3] The methyl ester of β-apocarotenoic acid is assayed for purity by chromatography on a column of partially deactivated aluminum oxide or by chromatography on a thin layer of secondary magnesium phosphate or alkaline silica gel G (Merck). The thin-layer chromatograms are developed with a mixture of petroleum ether–ethyl ether (19:1).

*Visible Spectrum.* Petroleum ether: 446 and 471 nm. $E^{1\%}_{1\,cm}$ 2575 and 2160, respectively.[3]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of the methyl ester has been reported.[4]

*Melting Point.*[3] The methyl ester of β-apocarotenoic acid melts at 136–137 °C.

**Probable Impurities:** Traces of *cis*-isomers of β-apocarotenoic acid methyl ester.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**


1. O. Wiss and H. Thommen, *Wiss. Veroeffentl. Deut. Ges. Ernaehrung*, 9, 188 (1963).
2. H. Thommen, *Chimia*, 15, 433 (1961).
3. O. Isler, W. Guex, R. Rüegg, G. Ryser, G. Saucy, U. Schwieter, M. Walter, and A. Winterstein, *Helv. Chim. Acta*, 42, 864 (1959).
4. U. Schwieter, G. Englert, N. Rigassi, and W. Vetter, *Pure Appl. Chem.*, 20, 365 (1969).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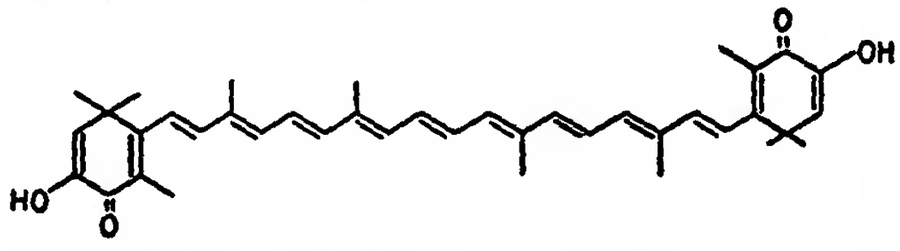
## Carot-5
## Astacin
## 3,3′-Dihydroxy-2,3,2′,3′-tetrahydro-β,β-carotene-4,4′-dione ≒ β,β-Carotene-3,4,3′,4′-tetrone (Astacene)

**Formula:** $C_{40}H_{52}O_4$
**Formula Wt.:** 592.83
**Calc. %:** C, 81.04; H, 8.16; O, 10.80



Astacin has been characterized as 3,3′,4,4′-tetraketo-β-carotene. The enol form of this compound (shown above) preponderates.[1]

**Sources:**

*Natural Sources.* Astacin is not a commonly occurring natural pigment. However, it may be obtained on treatment of astaxanthin or astaxanthin esters with alkali.[2] Astaxanthin may be isolated from some algae and from a wide variety of animals.[3] One of the best sources of this compound is lobster shells.[4]

*Chemical Synthesis.* The synthesis of astacin from canthaxanthin has been reported.[8]

**Isolation Procedures:**[3,4] Shells of freshly killed lobsters are covered with 2 M HCl and left to stand therein until they turn red. They are then washed with water and the hypodermis is separated. Pigment is extracted with acetone at room temperature and then transferred to petroleum ether, with dilution of the extract with water. The petroleum ether solution is washed with water and 90% methanol, diluted with 2 M NaOH and sufficient ethanol to form a homogeneous solution, and kept in the dark at room temperature for 5 h. Sufficient water is then added to produce two layers. The ethanolic layer is separated, and covered with petroleum ether, and the astacene is precipitated by careful acidification with acetic acid. The pigment is washed with hot water, dissolved in a small volume of highly purified pyridine, and crystallized by the addition of a small proportion of water.

**Methods of Purification:**

*Chromatography.* Astacin may be chromatographed on such weak neutral adsorbents as alumina–fibrous clay[4] (1:4) or sucrose.[8] Other adsorbents, such as alumina, adsorb this compound too tightly, whereas such compounds as calcium carbonate do not adsorb it.

*Solvent Partition.* Astacin may be partially purified by partition between petroleum ether and alkaline methanol.[1,6]

*Crystallization.* Astacin crystallizes in needles from pyridine–water.[4]

*Derivatives.* Astacin forms a dioxime,[7] a bis-phenazine derivative,[7] and such esters as the diacetate[1,5,6] and dipalmitate.[2]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of astacin may be determined by chromatography on kieselguhr paper.[1]

*Visible Spectrum.* Carbon disulfide,[2] broad maximum at 510 nm. Pyridine,[4] broad maximum at 500 nm. The $E^{1\%}_{1\,cm}$ (max), at 498 nm in pyridine is $1 \times 10^6$.[5]

*Infrared Spectrum.* The spectra for astacin and astacin diacetate in a potassium bromide pellet have been reported.[1]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of astacin has been reported.[1,8,8,9]

*Mass Spectrum.* The mass spectra of astacin and some of its derivatives have been determined.[10]

*Melting Point.* Several different melting points have been reported for this compound. These are 240–243 °C,[4] 241 °C,[11] 228 °C,[3] and 228–230 °C.[5]

*Optical Rotation.* Astacin is optically inactive.

**Probable Impurity:** Astaxanthin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**


1. A. J. Aasen and S. Liaaen-Jensen, *Acta Chem. Scand.*, 20, 1970 (1966).
2. R. Kuhn, J. Stene, and N. A. Sörensen, *Chem. Ber.*, 72, 1688 (1939).
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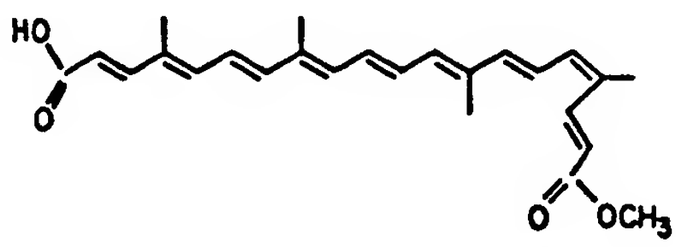
4. R. Kuhn and E. Lederer, *Chem. Ber.*, 66, 488 (1933).
5. J. B. Davis and B. C. L. Weedon, *Proc. Chem. Soc.*, 182 (1960).
6. R. Kuhn, E. Lederer, and A. Deutsch, *Z. Physiol. Chem.*, 220, 229 (1933).
7. P. Karrer and L. Loewe, *Helv. Chim. Acta*, 17, 745 (1934).
8. B. C. L. Weedon, in *Chemistry and Biochemistry of Plant Pigments*, T. W. Goodwin, ed., Academic Press, New York (1965).
9. B. C. L. Weedon, *Fortschr. Chem. Org. Naturstoffe*, 27, 81 (1969).
10. J. Baldas, Q. N. Porter, A. P. Leftwick, R. Holzel, B. C. L. Weedon, and J. Szabolcs, *J. Chem. Soc. (D)*, 415 (1969).
11. P. Karrer and F. Benz, *Helv. Chim. Acta*, 17, 412 (1934).


## Carot-6
## Bixin
## Methyl Hydrogen 9′-*cis*-6,6′-Diapocar tene-6,6′-dioate

**Formula:** $C_{25}H_{30}O_4$
**Structure:** See Kuhn and Winterstein,[1] Barber *et al.*[2,3]
**Formula Wt.:** 394.52
**Calc. %:** C, 76.11; H, 7.66; O, 16.23




**Sources:**

*Natural Sources.* Bixin has been isolated from the seeds of *Bixa orellana*[4] and all-*trans*-bixin has been found in the roots f *Aristolochia cymbifera*.[5] Bixin is extracted from commercial orlean, paté de rocou,[6] or bixa seeds[7] with organic solvents.

*Chemical Synthesis.* Methyl-*cis*-4-(natural) bixin[6,9] and all-*trans*-methyl bixin have been synthesized.[10–18]

**Methods of Purification:** Purification is achieved by recrystallization.

**Methods of Assaying for Purity:**

*Chromatography.* Methyl bixin and its geometrical isomers have been separated on chromatographic columns.[14] However, the column-chromatographic separation of the corresponding bixin isomers has not been reported. Methyl bixin has also been assayed for purity by thin-layer chromatography.[15]

*Derivatives.* Methyl bixin, norbixin, their dihydro and perhydro derivatives, and various other esters of bixin have been prepared.[4]

*Visible Spectrum.* Bixin:[4] carbon disulfide, 459, 489, and 523.5 nm; chloroform, 439, 469.5, and 503 nm; all-*trans*-bixin:[4] carbon disulfide, 457, 491, and 526.5 nm; chloroform, 443, 475, and 509.5 nm. Methyl bixin: benzene, 444, 471, and 502 nm. Absorption spectra for bixin in ethanol and methyl bixin in hexane have been reported.[6]

*Infrared Spectrum.* The infrared spectrum of methyl bixin has been reported.[16]

*Mass Spectrum.* The mass spectrum of bixindial has been reported.[17]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectra for methyl natural bixin,[3] all-*trans*-methyl bixin,[1,3] and *cis*-apo-1-norbixinal methyl ester[2,3] have been reported.

*Melting Point.* Bixin melts at 198 °C, all-*trans*-bixin[18] at 216–217 °C, and methyl bixin[4] at 163 °C.

**Probable Impurity:** all-*trans*-Bixin.

**Conditions of Storage:** The conditions of storage that are used for other carotenoids should be used for bixin. However, the compound is much more stable than many other carotenoids to air, heat, and light.[19]

**References**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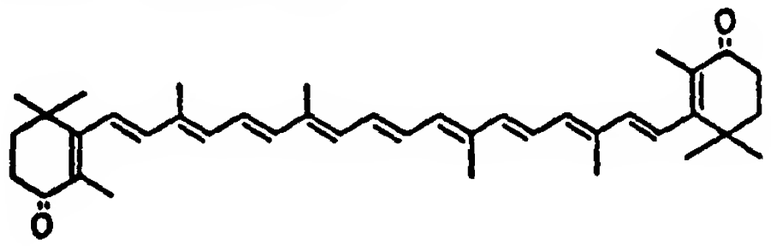
## Carot-7
## Canthaxanthin
## β,β-Carotene-4,4′-dione
## (β-Carotene-4,4′-dione)

**Formula:** $C_{40}H_{52}O_2$
**Formula Wt.:** 564.86
**Calc. %:** C, 85.06; H, 9.28; O, 5.66



**Sources:**

*Natural Sources.* Canthaxanthin has been reported to be present in crustaceae,[1] fishes (trout),[2] the organs of flamingos,[3,4] the feathers of several species of birds,[5–7] mushrooms,[8] insects,[9] and algae.[10]

*Chemical Synthesis.* Canthaxanthin has been synthesized from β-carotene,[11–13] 15,15′-didehydro-β-carotene,[14] crocetindialdehyde,[15] and dehydrocrocetindialdehyde.[16]

**Methods of Purification:** Canthaxanthin is separated from other pigments by chromatography on columns of magnesia or partially deactivated alumina. Further purification is achieved by recrystallization (dichloromethane or other solvents).

**Methods of Assaying for Purity:**

*Chromatography.* Purity of the pigment may be determined by chromatography on columns of deactivated alumina or magnesia, or by chromatography on a thin layer of silica gel G (Merck).[17] Dichloromethane–ethyl ether (9:1) is used to develop the chromatogram.

*Visible Spectrum.* Cyclohexane: The principal light-absorption maximum is found at 470 nm. An $E^{1\%}_{1\,cm}$ value of 2200 is found at this wavelength.

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of canthaxanthin has been reported.[18,19]

*Mass Spectrum.* The mass spectrum of canthaxanthin has been reported.[20,21]

*x-Ray Crystallography.* The configuration of canthaxanthin has been determined.[22]

*Melting Point.* A melting point of 211–212 °C has been reported.[16]

**Probable Impurities:** Echinenone and *cis*-isomers.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

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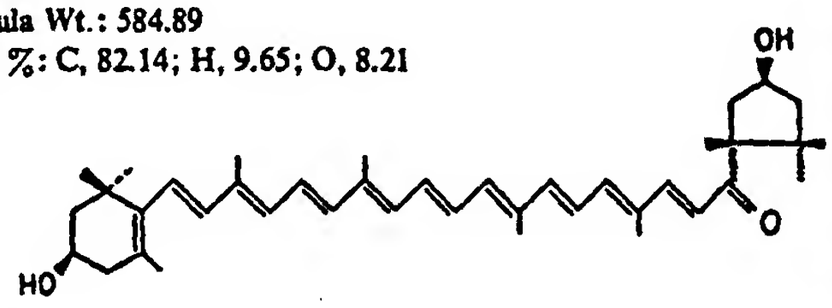
## Carot-8
## Capsanthin
## (3*R*,3′*S*,5′*R*)3,3′-Dihydroxy-β,κ-caroten-6′-one

**Formula:** $C_{40}H_{56}O_4$
**Formula Wt.:** 584.89
**Calc. %:** C, 82.14; H, 9.65; O, 8.21



**Sources:**

*Natural Sources.* Fruits of red peppers (*Capsicum annum*) are a good source of capsanthin.[1–3]

*Chemical Synthesis.* The structure of capsanthin has been established through degradation,[4–6] and its synthesis from β-citraurin has been reported.[7] The absolute configuration of capsanthin has been established as 3*R*, 3′*S*, 5′*R*.[8,9,10]

**Methods of Purification:** The extraction, chromatography on calcium carbonate, and crystallization of capsanthin have been reported.[1,11]

**Methods of Assaying for Purity:**

*Chromatography.* Capsanthin may be assayed for purity by chromatography on columns of calcium carbonate or zinc carbonate. Carbon disulfide or benzene-ethyl ether is used to develop the column.[1,12] Purity may also be determined by chromatography on a thin layer of silica gel G ($R_f$ 0.16 in a system of 20% ethyl acetate in dichloromethane),[13] or kielselguhr impregnated with vegetable oil ($R_f$ 0.74 in a system of 20:4:3 methanol–acetone–water, saturated with vegetable oil).[14]

*Derivatives.* The following derivatives of capsanthin have been prepared: capsanthin diiodide; capsanthol; capsanthin diacetate, dipropionate, dibutyrate, divalerate, dicaproate, dicaprate, dimyristate, dipalmitate, distearate, and dibenzoate; capsanthone;

anhydrocapsanthone; capsanthylal; capsanthylal monoxime; and capsaldehyde.[15,16]

*Solvent Partition.* The solvent-partiti n rati between petroleum ether and 90% methanol is 0:100.[15]

*Visible Spectrum.* Carbon disulfide: 503 and 542 nm. Petroleum ether: 475 and 505 nm. Benzene: 486 and 520 nm, $E^{1\%}_{1cm}$ 1790 at 486 nm.[16] Complete spectrum.[16–17] Iodine-isomerized capsanthin sh ws a "*cis* peak" at 355 nm.

*Infrared Spectrum.* The infared spectrum of capsanthin has been reported.[18]

*Mass Spectrum.* The mass spectrum of capsanthin has been reported.[19]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of capsanthin has been reported.[20]

*Optical Rotatory Dispersion.* The optical rotatory dispersion curves of capsanthin and related compounds have been reported.[16]

*Melting Point.* The melting point of capsanthin has been reported to be 176 °C (uncorrected)[21] and 175–176 °C (corrected).[11]

*Optical Rotation.* An $[\alpha]_{Cd}$ of +36° (chloroform) has been reported.[15]

**Probable Impurities:** *cis*-Isomers and traces of zeaxanthin and capsorubin.[15]

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20° C).

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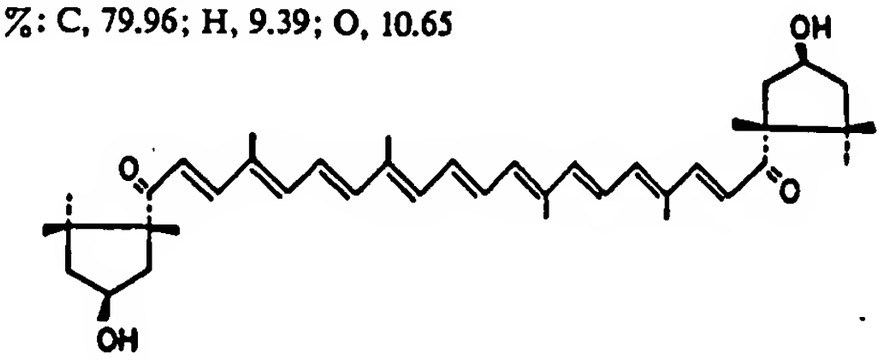
## Carot-9
## Capsorubin
## (3*S*,5*R*,3′*S*,5′*R*)3,3′-Dihydroxy-κ,κ-carotene-6,6′-dione

**Formula:** $C_{40}H_{56}O_4$
**Formula Wt.:** 600.89
**Calc. %:** C, 79.96; H, 9.39; O, 10.65




**Sources:**

*Natural Sources.* Capsorubin occurs as an ester in ripe paprika (*Capsicum annum*) fruits.[1,2] The absolute configuration of capsorubin has been established as 3*S*, 5*R*, 3′*S*, 5′*R*.[3,4,5,6]

*Chemical Synthesis.* The chemical synthesis of capsorubin has been reported.[3]

**Isolation Procedures:**[1,7] Paprika pods are pretreated with ethanol, and then extracted with petroleum ether. The combined extracts are concentrated in vacuum, and the residue is chromatographed on calcium carbonate. The capsorubin ester is saponified with methanolic potassium hydroxide. The unsaponifiable fraction, in carbon disulfide solution, is rechromatographed on calcium carbonate. The chromatographically purified pigment is then crystallized from benzene–petroleum ether.

**Methods of Purification:**

*Chromatography.* Capsorubin may be chromatographed on a column of calcium carbonate[7] or magnesia.[8]

*Solvent Partition.* A partial purification of capsorubin may be achieved by solvent partition.[7,8]

*Crystallization.* Capsorubin crystallizes as violet needles from benzene–petroleum ether and as plates from carbon disulfide.[7]

*Derivatives.* A number of esters of capsorubin have been prepared. These are the diacetate,[7,8] dipropionate, dibutyrate, divalerate, dicapronate, dicaprinate, dimyristate, dipalmitate, and distearate.[8] The reduction product, capsorubol,[8,10,11] and the oxidation product, capsorubone,[12–14] have also been prepared.

**Methods of Assaying for Purity:**

*Chromatography.* The homogeneity of capsorubin preparations can be ascertained by microchromatographic methods.[15,16] Chromatography on circular paper impregnated with a kieselguhr filler is recommended.[16]

*Visible Spectrum.* Hexane:[8] 443, 468, and 503 nm. Petroleum ether:[7] 444, 474, and 507 nm. Benzene: 455, 486, and 520 nm;[7,17] 460, 487, and 522 nm;[8] 463, 487, and 522 nm.[14] Carbon disulfide:[7] 468, 502, and 541.5 nm. Ethanol:[8] 482 nm. Spectral curves of capsorubin in hexane, benzene, and ethanol have been reported.[8] Molar absorption coefficients in benzene are the following:[14] 463 nm, $89.2 \times 10^3$; 487 nm, $129.8 \times 10^3$; and 522 nm, $119.1 \times 10^3$.

*Infrared Spectrum.* The infrared spectrum of capsorubin in chloroform has been reported.[11]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of capsorubin has been reported.[18,18]

*Mass Spectrum.* The mass spectrum of capsorubin has been reported.[19,20]

*Melting Point.*[9] Capsorubin melts at 218 °C.

*Optical Rotation.* The optical rotatory dispersion curve of capsorubin has been reported.[6]

**Probable Impurities:** Zeaxanthin and capsanthin.

Conditions of Storage: Darkness (brown vial), inert atmosphere (sealed ampoule), and l w temperature (−20 °C).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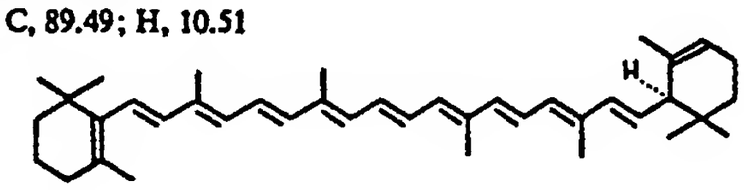
## Carot-10
## α-Carotene
## (6′*R*)β,ε-Carotene

Formula: $C_{40}H_{56}$
Formula Wt.: 536.89
Calc. %: C, 89.49; H, 10.51



Sources:

*Natural Sources.* α-Carotene is present in much smaller proportions than β-carotene in most plant species. However, the best sources of this carotene are the same as the sources of β-carotene: carrots,[1] palm oil,[2,3] and green leaves of various species.[4] In some algae, α-carotene is the major carotene.[5]

*Chemical Synthesis.* The chemical synthesis of α-carotene has been reported.[6–9] The absolute configuration of α-carotene has been established as 6′*R*.[10]

Isolation Procedures: The extraction of α-carotene from plant sources, and its purification by distribution between immiscible solvents, chromatography, and crystallization have been reported.[1–4,11]

Methods of Purification:

*Chromatography.* α-Carotene has been purified by chromatography on columns of calcium hydroxide, alumina, or magnesia.[12]

*Crystallization.* α-Carotene may be crystallized from the same solvent pairs used to crystallize β-carotene and lycopene.[12]

Methods of Assaying for Purity:

*Chromatography.* The purity of α-carotene may be determined by chromatography on columns,[12] alumina paper,[13] or thin-layer plates of magnesia.[14]

*Solvent Partition.*[15] The partition ratio between hexane and 95% methanol is 100:0.

*Visible Spectrum.* Hexane (b.p. 65–67 °C): 422, 446, and 474 nm. $E^{1\%}_{1\,cm}$ 2725 (446 nm) and 2490 (474 nm). Spectral curve.[12] Light petroleum: 422, 444, and 473 nm. $E^{1\%}_{1\,cm}$ 2800 (444 nm).

*Mass Spectrum.* The mass spectrum of α-carotene has been reported.[16–19]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of α-carotene has been reported.[16,17,20]

*Optical Rotatory Dispersion.* The optical rotatory dispersion curve of α-carotene has been reported.[21]

*Melting Point.*[1,4] α-Carotene melts at 184–188 °C.

*Optical Rotation.* $[\alpha]^{15}_{436}$ +385° has been reported.[1]

Probable Impurities: Oxidation products, *cis*-isomers, and other carotenes (β-carotene and phytofluene).

Conditions of Storage: Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).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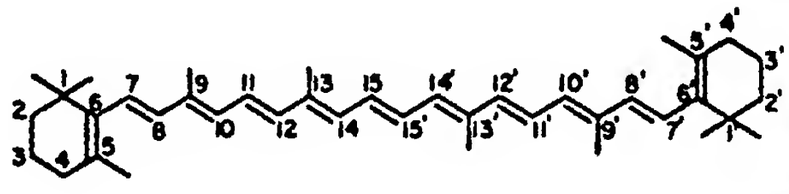
19. C. R. Enzell, G. W. Francis, and S. Liaaen-Jensen, *Acta Chem. Scand.*, 23, 727 (1969).
20. B. C. L. Weedon, *Fortschr. Chem. Org. Naturstoffe*, 27, 81 (1969).
21. L. Bartlett, W. Klyne, W. P. Mose, P. M Scopes, G. Galasko, A. K. Mallams, B. C. L. Weedon, G. Szabolcs, and G. Tóth, *J. Chem. Soc.* (*C*), 2527 (1969).


## Carot-11
## β-Carotene
## β,β-Carotene

Formula: $C_{40}H_{56}$
Formula Wt.: 536.89
Calc. %: C, 89.49; H, 10.51



**Sources:**

*Natural Sources.* Excellent sources are carrots,[1] palm oil,[2] and green leaves of many plant species.[3]

*Chemical Synthesis.* Several chemical syntheses f β-car tene have been reported.[4–7]

**Isolation and Methods of Purification:** The extraction f β-carotene from plant material and the separation and purification of this compound by solvent partition, chromatography, and crystallization have been reported.[1–3,8,9] The standardization of some of the steps in these procedures has been effected through cooperative studies.[10]

**Methods of Assaying for Purity:**

*Chromatography.* Purity of the compound may be determined by column chromatography (usually on magnesia),[10] chromatography on kieselguhr paper,[11] or chromatography on a thin layer of magnesia.[12]

*Solvent Partition.*[13] The partition ratio between hexane and 95% methanol is 100:0.

*Visible Spectrum.*[4,8,14] Hexane (b.p. 65–67 °C): 450 and 478 nm. $E^{1\%}_{1\,cm}$ 2590 and 2280. Cyclohexane: 456 and 484 nm. $E^{1\%}_{1\,cm}$ 2500 and 2150.

*Infrared Spectrum.* The infrared absorption spectrum has been reported.[6]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum has been reported.[15]

*x-Ray Crystallography.* The configuration of β-carotene has been determined.[16]

*Mass Spectrum.* The mass spectrum of β-carotene has been reported.[17–20]

*Melting Point.* β-Carotene melts at 178–180 °C.[2,8]

**Probable Impurities:** Oxidation products and *cis*-isomers; small proportions of related carotenes (α- and ζ-carotenes) may also be present if chromatographic purification has not been properly performed.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), low temperature (−20 °C).

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# Carot-12
# γ-Carotene
# β,ψ-Carotene

**Formula:** $C_{40}H_{56}$
**Formula Wt.:** 536.89
**Calc. %:** C, 89.49; H, 10.51

**Sources:**

*Natural Sources.* A strain of *Penicillium sclerotiorum* is one f the best sources of this pigment.[1] This compound is also present in small proportions in many plant materials, particularly fruits, that contain β-carotene.[2]

*Chemical Synthesis.* The chemical synthesis of γ-carotene has been reported by Garbers *et al.*[3] and Rüegg *et al.*[4]

**Isolation Procedures:** γ-Carotene is isolated from plant materials by the methods of solvent extraction, saponification, phasic separation, and chromatography commonly used for other carotenes.[2]

**Methods of Purification:**

*Solvent Partition.* γ-Carotene is epiphasic on partition between 90% aqueous methanol and petroleum ether.[2]

*Chromatography.* Purification of γ-carotene may be achieved by chromatography on a column of aluminum oxide[4,6] or calcium hydroxide.[5]

*Crystallization.* Benzene–methanol (2:1) has been used to crystallize γ-carotene.[5]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of γ-carotene may be determined by column (aluminum oxide) or thin-layer (magnesium oxide) chromatography.[7]

*Visible Spectrum.*[2,4,7] Petroleum ether: 437, 462, and 494 nm. $E^{1\%}_{1\,cm}$ 2055, 3100, and 2720, respectively. Benzene: 448, 477, and 510 nm.

*Infrared Spectrum.* The infrared spectrum of γ-carotene has been reported.[7]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of γ-carotene has been reported.[7–9]

*Mass Spectrum.* The mass spectrum of γ-carotene has been reported.[7,8]

*Melting Point.* A melting point of 150 °C has been reported[10] for natural γ-carotene. Synthetic *trans*-γ-carotene melts[4,7] at 152–154 °C.

**Probable Impurities:** Oxidation products, *cis*-isomers, and lycopene (when isolated from natural sources).

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

**References**


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## Carot-13
## ζ-Carotene
## 7,8,7′,8′-Tetrahydro-ψ,ψ-carotene
## (7,8,7′,8′-Tetrahydrolycopene)

**Formula:** $C_{40}H_{60}$
**Formula Wt.:** 540.90
**Calc. %:** C, 88.82; H, 11.18

**Sources:**

*Natural Sources.* Good sources of ζ-carotene are carrot roots,[1] fruit of certain varieties of tomato,[2] fruit of *Lonicera japonica*, the petals of *Calendula officinalis*, and certain fungi.[3] An unsymmetrical ζ-carotene (7,8,11,12-tetrahydrolycopene) is found in several photosynthetic bacteria.[4]

*Chemical Synthesis.* The chemical synthesis of the symmetrical and unsymmetrical ζ-carotenes has been reported.[5,6]

**Isolation Procedures:** ζ-Carotene is isolated from plant materials by the methods of solvent extraction, saponification, phasic separation, and chromatography commonly used for other carotenes.[7]

**Methods of Purification:**

*Solvent Partition.* ζ-Carotene is epiphasic on partition between 90% aqueous methanol and petroleum ether.

*Chromatography.* ζ-Carotene may be purified from other carotenes by chromatography on 50% magnesia–Hyflo Supercel.[7] Columns are developed with hexane, and ζ-carotene is eluted with 10% ethanol in hexane. Purification of ζ-carotene may also be effected on a column of alumina.[7] The chromatogram is developed with hexane containing 2–5% of diethyl ether.

*Crystallization.* The crystallization of ζ-carotene has been reported.[5]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of ζ-carotene may be determined by column[7] or thin-layer[8] chromatography.

*Visible Spectrum.* Petroleum ether: 378, 400, and 425 nm.[5,7,8] $E^{1\%}_{1\,cm}$ 2270 (400 nm).[5,7,8] Carbon disulfide:[9] 402, 424, and 452 nm. The unsymmetrical ζ-carotene (7,8,11,12-tetrahydrolycopene) in petroleum ether has wavelength maxima[4] at 374, 394.5, and 418.5 nm.

*Infrared Spectrum.* The infrared spectra for the two forms of ζ-carotene have been reported.[5,6,10]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectra of ζ-carotene and its unsymmetrical isomer have been reported.[5,6]

*Mass Spectrum.* The mass spectra of symmetrical and unsymmetrical ζ-carotene have been reported.[4,11,12]

*Melting Point.* A melting-point range of 38–42 °C has been reported for all-*trans*-ζ-carotene.[5,6]

**Probable Impurities:** Oxidation products.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed vial), and low temperature (−20 °C).[9]

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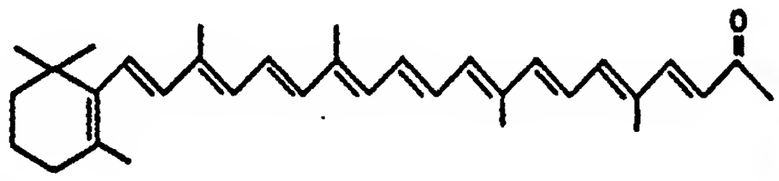
## Carot-14
## Citranaxanthin
## 5′,6′-Dihydro-5′-apo-18′-nor-β-caroten-6′-one

**Formula:** $C_{32}H_{44}O$
**Formula Wt.:** 456.72
**Calc. %:** C, 86.79; H, 9.71; O. 3.50



**Sources:**

*Natural Sources.* Citranaxanthin is found in the peel of the trigeneric hybrid *Sinton citrangequat*.[1]

*Chemical Synthesis.* The chemical synthesis of citranaxanthin from β-apo-8′-carotenal ($C_{30}$) and acetone has been reported.[1]

**Isolation Procedures:** The extraction of citranaxanthin from the peel of *Sinton citrangequat* fruit, the partial purification of this compound by distribution between immiscible solvents and its complete purification by chromatography and crystallization have been reported.[1]

**Methods of Purification:**

*Chromatography.* Citranaxanthin is purified by chromatography on a column of 1:1 (w/w) magnesium oxide–Hyflo Supercel.[1]

*Crystallization.* Citranaxanthin has been crystallized from petroleum ether.[1]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of citranaxanthin may be determined by column chromatography[1] or by chromatography on thin-layer plates of silica gel G. The chromatoplates are developed with 2:3 petroleum ether–benzene.

*Visible Spectrum.* Hexane: 349 and 466 nm; $E^{1\%}_{1\,cm}$ 410 and 2575, respectively. Cyclohexane: 352 and 471 nm; $E^{1\%}_{1\,cm}$ 400 and 2475, respectively. Benzene: 360 and 482 nm; $E^{1\%}_{1\,cm}$ 365 and 2275, respectively.[1]

*Infrared Spectrum.* The infrared absorption spectrum of citranaxanthin has been determined.[1]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of citranaxanthin has been determined.[1]

*Melting Point.*[1] Citranaxanthin melts at 155–156 °C.

*Derivatives.*[1] Citranaxanthin f rms an oxime that melts at 196–197 °C.

**Probable Impurities:** Oxidation products and *cis*-isomers.

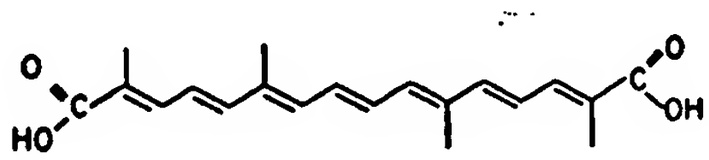
**Conditions of Storage:** Darkness (br wn vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

**Reference**

1. H. Yokoyama and M. J. White, *J. Org. Chem.*, 30, 2481 (1965).

## Carot-15
## Crocetin
## 8,8′-Diapocarotene-8,8′-dioic Acid

**Formula:** $C_{20}H_{24}O_4$
**Formula Wt.:** 328.41
**Calc. %:** C, 73.13; H, 7.37; O, 19.51



**Sources:**

*Natural Sources.* Crocetin (unesterified) is present in trace amounts in some plant species. Most of this compound is found as digentiobiose ester (crocin).[1–3] Crocetin has been isolated as the dimethyl ester; this compound is formed through the action of dilute sodium hydroxide in methanol on an extract of saffron, *Crocus sativus.*[4] Crocin is also found in *Cedrela toona*, *Nyctanthes arbor-tristis*, and *Verbascum phlomoides* petals and in the fruit of *Gardenia grandiflora.*[5]

*Chemical Synthesis.* The total synthesis of all-*trans*-crocetin dimethyl ester has been reported.[6,7]

**Methods of Purification:** Crocetin from saffron[8] and from the petals f *Crocus luteus*,[8] crocin from saffron,[9] and crocetin dimethyl ester from saffron[10] are purified largely by chromatography followed by crystallization, usually as the dimethyl ester.

**Methods of Assaying for Purity:**

*Chromatography.* Crocetin dimethyl ester may be assayed for purity by column chromatography.[11]

*Derivatives.* Mono- and dimethyl esters of crocetin, perhydrocrocetin and its dimethyl ester and diamide, dihydrocrocetin and its dimethyl ester, and crocetin tetrabromide have been prepared.[8]

*Visible Spectrum.*[8] Crocetin, in carbon disulfide: 426, 453, and 482 nm; pyridine: 411, 436, and 464 nm; chloroform: 434.5 and 463 nm; petroleum ether (b.p. 40–60 °C): 424.5 and 450.5 nm; hexane: 400, 420, and 444.5 nm. Crocetin dimethyl ester–petroleum ether (b.p. 40–60 °C): 422 and 448 nm;[6] 422 and 450 nm;[7] 420 and 444.5 nm.[10] $E^{1\%}_{1\,cm}$ values are given as follows: Crocetin dimethyl ester in petroleum ether, 4750 at 448 nm; in ethanol,[8] 5000 at 425 nm.

*Infrared Spectrum.* The infrared spectrum of crocetin dimethyl ester has been reported.[12,13]

*Mass Spectrum.* The mass spectrum of crocetindial has been reported.[14]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectra of crocetin dimethyl ester and crocetindial have been reported.[15]

*Melting Point.* The following melting points have been reported: crocetin, 285 °C; and crocetin dimethyl ester,[16] 223 °C.

**Probable Impurity:** Picrocrocin, a colorless glycoside closely related t crocin.

**Conditions of Storage:** Darkness (br wn vial), inert atmosphere (sealed ampoule), and l w temperature (−20 °C).

**References**

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2. P. Karrer and H. Salomon, *Helv. Chim. Acta*, 11, 513 (1928).
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15. B. C. L. Weedon, *Fortschr. Chem. Org. Naturstoffe*, 27, 81 (1969).
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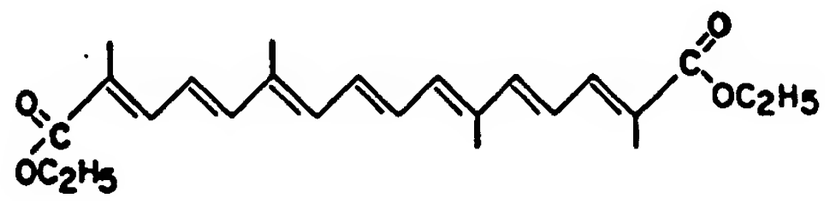
## Carot-16
## Crocetin Diethyl Ester
## Diethyl 8,8′-Diapocarotene-8,8′-dioate

**Formula:** $C_{24}H_{32}O_4$
**Formula Wt.:** 384.52
**Calc. %:** C, 74.97; H, 8.39; O, 16.64



**Sources:**

*Natural Sources.* Crocetin is present as the digentiobiose ester (crocin) in saffron (*Crocus sativus*) flowers.[1–4]

*Chemical Synthesis.* The chemical synthesis of crocetin diethyl ester has been reported.[5,6]

**Isolation Procedures:**[7] Crocin and crocetin are normally extracted from dried saffron flowers with ethanol after a pre-extraction of the flowers with ether. Crocin is obtained by crystallization from the alcoholic extract by the addition of ether. Crystalline crocetin may be obtained by addition of ether to an alcoholic extract f saffron flowers after prior saponification and acidification.

**Methods of Purification:**

*Chromatography.* Crocetin diethyl ester may be purified by chromatography on a column of silica gel G.[7]

*Crystallization.* Crocetin diethyl ester has been crystallized from benzene.[5]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of crocetin diethyl ester may be determined by chromatography on a column or thin-layer plate f silica gel G. Dichloromethane is the solvent used in these separations.[8]

*Visible Spectrum.* Petroleum ether: 400, 422, and 450 nm. $E^{1\%}_{1\,cm}$ 2340, 3820, and 3850, respectively.[5] Cyclohexane: 402, 425,

and 452 nm. $E^{1\%}_{1\,cm}$ 2190, 3590, and 3640, respectively. Benzene: 411, 435, and 462 nm. $E^{1\%}_{1\,cm}$ 1970, 3110, and 3000, respectively. Chl rof rm: 412, 434, and 462 nm. $E^{1\%}_{1\,cm}$ 2190, 3365, and 3200, respectively.[6]

*Melting Point.* Melting points of 218–219 °C and 216–218 °C have been reported[6] for crocetin diethyl ester.

**Probable Impurities:** Oxidation products and *cis*-isomers.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

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5. O. Isler, H. Gutmann, M. Montavon, R. Rüegg, G. Ryser, and P. Zeller, *Helv. Chim. Acta*, 40, 1242 (1957).
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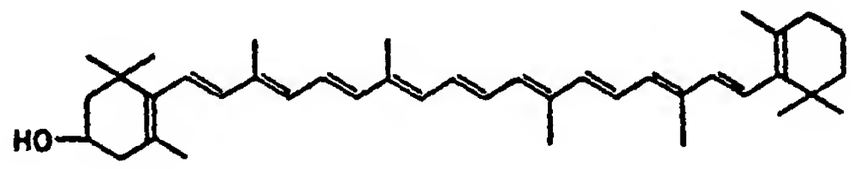
## Carot-17
## Cryptoxanthin
## (3*R*)β,β-Caroten-3-ol
## (β-Caroten-3-ol)

**Formula:** $C_{40}H_{56}O$
**Formula Wt.:** 552.89
**Calc. %:** C, 86.90; H, 10.21; O, 2.89




**Sources:**

*Natural Sources.* Cryptoxanthin may be isolated from maize seeds[1] or calyces of *Physalis alkekengi.*[2] It also occurs in some fruits[3] and in milk and butter.[4] The absolute configuration of cryptoxanthin has been established[5,6] as 3*R*.

*Chemical Synthesis.* The synthesis of cryptoxanthin has been reported.[7,8]

**Isolation Procedures:** The extraction, chromatography, and crystallization of cryptoxanthin have been reported.[2,9,10]

**Methods of Purification:**

*Chromatography.* Cryptoxanthin may be purified by chromatography on magnesia, calcium carbonate, or deactivated alumina. Ethanol in ethyl ether is used to develop the column.[2]

*Crystallization.* Cryptoxanthin may be crystallized from a mixture of chloroform and ethanol.[2,7]

**Methods of Assaying for Purity:**

*Chromatography.* Purity of the compound may be determined by column chromatography on magnesia[2] or by chromatography on kieselguhr paper.[11]

*Solvent Partition.* The partition ratio between hexane and 90% methanol is 87:3.[11]

*Visible Spectrum.* Petroleum ether[7] (b.p. 40–60 °C): 452 and 480 nm. $E^{1\%}_{1\,cm}$ 2370 and 2080. Ethanol: 451.5 and 478 nm. $E^{1\%}_{1\,cm}$ 2460 and 2165. Hexane: 450 and 478 nm. $E^{1\%}_{1\,cm}$ 2460 at 450 nm.

*Infrared Spectrum.* The infrared spectrum of cryptoxanthin has been reported.[7]

*Optical Rotatory Dispersion.* The optical r tatory dispersion curve of cryptoxanthin has been reported.[9]

*Melting Point.* Melting points of 165–169 °C[2] and 158–160 °C[7] have been reported.

**Probable Impurities:** Oxidation products and *cis*-isomers.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), low temperature (−20 °C).

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## Carot-18
## 2,2′-Diketospirilloxanthin
## 1,1′-Dimethoxy-3,4,3′,4′-tetrahydro-1,2,1′,2′-tetrahydro-ψ,ψ-carotene-2,2′-dione

**Formula:** $C_{42}H_{58}O_4$
**Formula Wt.:** 624.91
**Calc. %:** C, 80.73; H, 9.03; O, 10.24


**Sources:**

*Natural Sources.* 2,2′-Diketospirilloxanthin has been isolated from *Rhodopseudomonas spheroides*[1] and *Rhodopseudomonas gelatinosa.*[1,3]

*Chemical Synthesis.* The chemical synthesis of 2,2′-diketospirilloxanthin has been reported.[3,4]

**Isolation Procedures:** 2,2′-Diketospirilloxanthin is extracted from *Rhodopseudomonas* species with acetone, the material is saponified (after removal of acetone), and the nonsaponifiable compounds are transferred to a benzene–petroleum ether mixture.[1,3,5]

**Methods of Purification:**

*Chromatography.* 2,2′-Diketospirilloxanthin is purified by chromatography on a column of partially deactivated, neutral aluminum oxide.[5]

*Crystallization.* 2,2′-Diketospirilloxanthin has been crystallized from acetone–petroleum ether.[5]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of 2,2′-diketospirilloxanthin may be determined by chromatography on filter paper containing a kieselguhr filler, by thin-layer chromatography on silica gel G

plates, r by chromatography on a column of partially deactivated, neutral aluminum xide.[3]

*Solvent Partition.*[5] The partition ratio between petroleum ether and 95% methanol is 41:9.

*Visible Spectrum.* Petr leum ether:[4] 487.5, 518, and 555 nm. Hexane: 349, 422, 488, 516, and 551 nm. $E^{1\%}_{1cm}$ 550, 820, 2125, 2725, and 2150, respectively. Cyclohexane: 352, 432, 495, 523, and 560 nm. $E^{1\%}_{1cm}$ 570, 800, 2055, 2580, and 2025, respectively. Benzene: 361, 539, and 576 nm. $E^{1\%}_{1cm}$ 550, 2365, and 1822, respectively. Carbon disulfide:[5] 530, 561, and 603 nm.

*Infrared Spectrum.* The infrared spectrum of 2,2′-diketospirilloxanthin has been determined.[5]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of 2,2′-diketospirilloxanthin has been determined.[1]

*Mass Spectrum.* The mass spectrum of 2,2′-diketospiril- l xanthin has been published.[6]

*Melting Point.* A melting point of 222 °C has been reported for naturally occurring 2,2′-diketospirilloxanthin, and of 225–227 °C f r the synthetic compound.[3]

**Probable Impurities:** Oxidation products and *cis*-isomers.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

**References**


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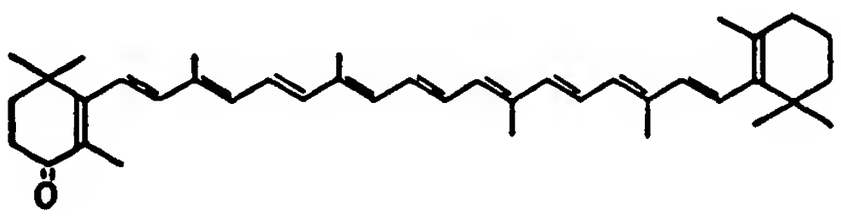
## Carot-19
## Echinenone
## β,β-Caroten-4-one
## (β-Caroten-4-one)

**Formula:** $C_{40}H_{54}O$
**Formula Wt.:** 550.88
**Calc. %:** C, 87.21; H, 9.88; O, 2.90



**Sources:**

*Natural Sources.* Echinenone is found in echinoideae,[1,2] in crustacea,[3] and in blue-green algae.[4]

*Chemical Synthesis,* Echinenone is prepared synthetically from β-apo-8′-carotenal (Carot-2).[5,6]

**Methods of Purification:** Echinenone is purified by column chromatography on partially deactivated alumina or magnesia.[1–3] Further purification is achieved through crystallization.

**Methods of Assaying for Purity:**

*Chromatography.* The purity of echinenone may be determined by chromatography on partially deactivated alumina or magnesia,[1,2] or by chromatography on a thin layer of silica gel G with 4:1 cyclohexane–ethyl ether as the developing solvent.

*Visible Spectrum.* The principal maximum is found at 472 nm (benzene), 461 nm (cyclohexane), and 458 nm (petroleum ether, b.p. 80–105 °C). The $E^{1\%}_{1cm}$ values are 2040, 2110, and 2160, respectively. Treatment of echinenone with sodium borohydride causes a shift in the absorption maximum (in ethan l) fr m 470 nm t 423, 451, and 478 nm.

*Mass Spectrum.* The mass spectrum of echinenone has been re- p rted,[7,8]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of echinenone has been reported.[9]

*Melting Point.* A melting point of 178–179 °C has been reported.[4]

**Probable Impurities:** Oxidation products and traces of *cis*-isomers.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**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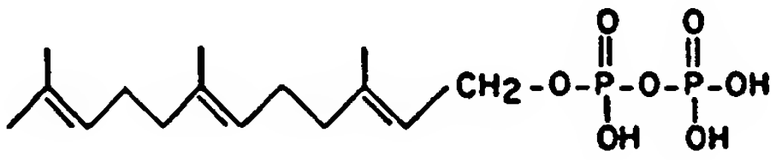
## Carot-20
## Farnesyl Pyrophosphate

**Formula:** $C_{15}H_{28}O_7P_2$
**Formula Wt:** 382.34
**Calc %:** C, 47.13; H, 7.38; O, 29.29; P, 16.20



**Sources:**

*Natural Sources.* Farnesyl pyrophosphate does not normally accumulate in significant quantity in biological materials. However, this compound has been synthesized enzymically from mevalonic acid[1] and from isopentenyl and dimethylallyl pyrophosphates.[2,3]

*Chemical Synthesis.* The chemical synthesis of farnesyl pyrophosphate is effected through the pyrophosphorylation of farnesol.[4–6]

**Methods of Purification:**

*Derivative Formation.* Chemical synthesis yields a mixture of farnesyl phosphate and farnesyl pyrophosphate. These compounds can be selectively crystallized by treatment of an aqueous solution of the mixture with cyclohexylamine followed by lithium chloride.[4,6] The products are the dicyclohexylammonium salt of farnesyl phosphate and the lithium salt of farnesyl pyrophosphate. The water-insoluble lithium salt can be converted into the ammonium salt by passage[6] through a column of Dowex-50 ion-exchange resin.

*Chromatography.* Farnesyl pyrophosphate may be purified[7] by chromatography on Whatman No. 3 MM paper in a system of (40:20:1:39) (v/v) isopropyl alcohol–isobutyl alcohol–amm nia–water. An $R_f$ value of 0.87 is obtained. The presence of phosphate

at this $R_f$ value is demonstrated by spraying with the Rosenberg reagent.[8]

**Methods of Assaying for Purity:**

*Phosphate Determination* Standard assays for the determination of phosphate are used t assay for the purity of farnesyl pyr phosphate.[5,6]

*Chromatography*. The purity of farnesyl pyrophosphate may be determined by paper[2,6] or ion-exchange[9] chromatography. Farnesyl pyrophosphate may be cleaved by treatment with 1 M HCl. The product (nerolidol) is extracted with petroleum ether, and assayed by gas-liquid chromatography.[1,10] Farnesyl pyrophosphate may also be cleaved by bacterial alkaline phosphatase or snake-venom diesterase. The liberated farnesol is extracted into petroleum ether and assayed by gas-liquid chromatography.[1,8,11]

*Enzymic Assay*. Radioactive farnesyl pyrophosphate may be converted into radioactive squalene by a liver enzyme system,[1,8] a yeast extract,[8] or a plant extract.[11] The radioactive squalene is extracted from the incubation mixture with petroleum ether, and then assayed by gas-liquid chromatography.

**Probable Impurities:** Geranyl pyrophosphate and farnesyl phosphate.

**Conditions of Storage:** Farnesyl pyrophosphate is stored as the lithium or ammonium salt at low temperature (0 °C).

**References**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3. J. K. Dorsey, J. A. Dorsey, and J. W. Porter, *J. Biol. Chem.*, 241, 5353 (1966).
4. F. Cramer and W. Böhm, *Angew. Chem.*, 71, 775 (1959).
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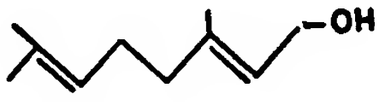
6. C. R. Childs, Jr., and K. Bloch, *J. Biol. Chem.*, 237, 62 (1962).
7. D. G. Anderson, M. S. Rice, and J. W. Porter, *Biochem. Biophys. Res. Commun.*, 3, 591 (1960).
8. H. Rosenberg, *J. Chromatogr.*, 2, 487 (1959).
9. R. E. Dugan, E. Rasson, and J. W. Porter, *Anal. Biochem.*, 22, 249 (1968).
10. L. A. Witting and J. W. Porter, *J. Biol. Chem.*, 234, 2841 (1959).
11. D. A. Beeler, D. G. Anderson, and J. W. Porter, *Arch. Biochem. Biophys.*, 102, 26 (1963).


## Carot-21
## Geraniol
## (3,7-Dimethyl-2,6-octadien-1-ol)

**Formula:** $C_{10}H_{18}O$
**Formula Wt.:** 154.24
**Calc. %:** C, 77.87; H, 11.76; O, 10.37




**Sources:**

*Natural Sources*. Geraniol is a constituent of many essential oils, such as palmarosa,[1] citronella,[2] ginger grass,[3] geranium,[4] and attar of roses.[5] It also occurs in oils of *Andropogon schoenanthus*,[1] *Pelargonium odoratissum*,[1] *Anthocephalus cadamba*,[6] *Artemisia campestris*,[6] citrus leaves,[6] carrots,[7] coriander,[8] lavender,[9] and *Juniperus sabina*.[10] It also is present in some algae[11] and seaweeds.[12] Geraniol may be obtained from citronella and other essential oils by fractional distillati n.

*Chemical Synthesis*. Geraniol has been synthesized by reduction of methyl geranate with lithium aluminum hydride.[13] It has als been synthesized in ether under pressure from 6-methyl-5-hepten-2-one, potassium hydroxide, and acetylene.[14] This reaction yields dehydrolinalool, which is then hydrogenated in the presence of palladium-on-calcium carbonate to linalool (96% yield), and this is converted into geraniol.[14]

**Methods of Purification:**

*Solvent Extraction*. The techniques of countercurrent distribuli n and liquid–liquid extraction have been used f r the isolati n of geraniol from geranium il.[3]

*Chromatography*. Geraniol may be purified by ascending paper chromatography[15] or by thin-layer chromatography on plates of kieselguhr G, with 130:70:1 acetone–water–liquid paraffin as the solvent system;[16] hexane–ethyl acetate (1:4) may also be used as a solvent system.[17] Geraniol may be purified by gas–liquid chromatography on a silicone-treated column of Carbowax 20 M (10%) on Chromosorb W (60–80 mesh).[18] Other gas–liquid chromatographic systems have been used.[10,18,20]

**Methods of Assaying for Purity:**

*Chromatography*. The aforementioned techniques of paper, thin-layer, and gas–liquid chromatography may be used t assay for the purity of geraniol.

*Derivatives*. Geraniol may be identified, and assayed for purity by preparation of the 3,5-dinitrobenzoate[21] (m.p. 63 °C), the phenylurethan[22] (m.p. 82 °C), or the allophanate[23] (m.p. 124–124.5 °C).

*Ultraviolet Spectrum*. The ultraviolet absorption maximum, in cyclohexane, is at 190–195 nm.[23]

*Infrared Spectrum*. The infrared spectrum of geraniol has been reported.[24]

*Nuclear Magnetic Resonance*. The nuclear magnetic resonance spectrum of geraniol has been reported.[25]

*Mass Spectrum*. The fragmentation pattern for geraniol has been reported.[26]

*Refractive Index*.[22] The $n_D^{20}$ is 1.4766.

*Density*.[22] The density of geraniol is 0.8894 g/ml at 20 °C.

*Solubility*. Soluble in alcohol and insoluble in water.[27]

*Boiling Point*.[22] Geraniol has been reported to boil at 230 °C.

*Probable Impurity*. The *cis*-isomer (nerol) is the most common impurity.

*Conditions of Storage*. Geraniol should be stored in full, tightly sealed containers, in a cool place, protected from light.

**References**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8. G. M. Makarova and Yu. G. Borisyuk, *Farmatsevt. Zh.* (Kiev), 14, 43 (1956); *Chem. Abstr.*, 58, 2320f (1963).
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10. E. V. Rudloff, *Can. J. Chem.*, 41, 2876 (1963).
11. T. Katayama, *Kagoshima Daigaku, Suisan Gakubu Kiyo*, 13, 58 (1964); *Chem. Abstr.*, 62, 10822h (1965).
12. T. Katayama, *Nippon Suisan Gakkaishi*, 27, 75 (1961); *Chem. Abstr.*, 56, 7710b (1962).
13. J. W. K. Burrell, R. F. Garwood, L. M. Jackman, E. Oskay, and B. C. L. Weedon, *J. Chem. Soc.*, 2144 (1966).
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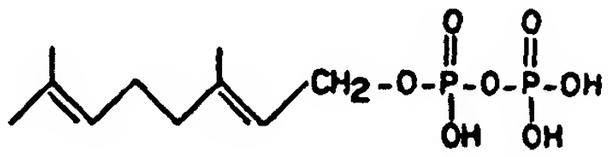
## Carot-22
## Geranyl Pyrophosphate

**Formula:** $C_{10}H_{20}O_7P_2$
**Formula Wt.:** 314.22
**Calc. %:** C, 38.23; H, 6.42; O, 35.64; P, 19.71



**Sources:**

*Natural Sources.* Geranyl pyrophosphate does not normally accumulate in significant quantity in biological material. Neither has an enzymic synthesis of geranyl pyrophosphate been developed that would result in the synthesis of an appreciable quantity of this compound.

*Chemical Synthesis.* The chemical synthesis of geranyl pyrophosphate is effected through the pyrophosphorylation of geraniol.[1]

**Methods of Purification:**

*Derivative Formation.* Chemical synthesis yields a mixture of geranyl phosphate and geranyl pyrophosphate. These compounds can be selectively crystallized by treatment of an aqueous mixture with cyclohexylamine followed by lithium chloride.[1] The products are geranyl phosphate dicyclohexylammonium salt and the lithium salt of geranyl pyrophosphate. The water-insoluble lithium salt can be converted into the ammonium salt by passage[2] through Dowex-50 ion-exchange resin.

*Chromatography.* Geranyl pyrophosphate may be purified by paper chromatography on Whatman No. 3 MM paper in a system of 40:20:1:39 (v/v) isopropyl alcohol–isobutyl alcohol–ammonia–water.[2,3] An $R_f$ value of 0.77–0.82 is obtained. The presence of phosphate at this $R_f$ may be shown by spraying with the Rosenberg reagent.[4]

**Methods of Assaying for Purity:**

*Phosphate Determination.* Standard assays for the determination of phosphate are used to assay for the purity of geranyl pyrophosphate.[5]

*Chromatography.* The purity of geranyl pyrophosphate may be determined by paper chromatography.[2,3] Geranyl pyrophosphate may be cleaved with acid, or with bacterial alkaline phosphatase, or snake-venom diesterase.[2,3] The resultant linalool or geraniol is extracted with petroleum ether, and assayed by gas–liquid chromatography.[3]



*Enzymic Assay.* A mixture of geranyl pyrophosphate plus isopentenyl pyrophosphate is converted into farnesyl pyrophosphate by farnesyl pyrophosphate synthetase.[2,3] The product of the reaction is treated with 1 M HCl or alkaline phosphatase, as mentioned, and the liberated terpenols are extracted with petroleum ether and assayed by gas–liquid chromatography.[3]

**Probable Impurity:** Geranyl phosphate.

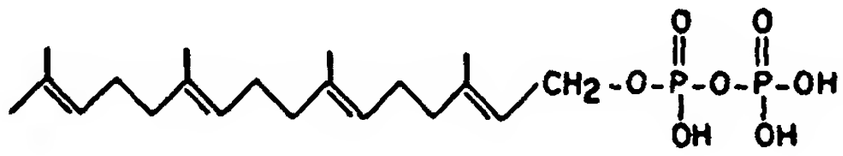
**Conditions of Storage:** Geranyl pyrophospate is stored as the lithium or ammonium salt at low temperature (0 °C).

**References**

1. F. Cramer and W. Böhm, *Angew. Chem.*, 71, 775 (1959).
2. C. R. Benedict, J. Kett, and J. W. Porter, *Arch. Biochem. Biophys.*, 110, 611 (1965).
3. J. K. Dorsey, J. A. Dorsey, and J. W. Porter, *J. Biol. Chem.*, 241, 5353 (1966).
4. H. Rosenberg, *J. Chromatogr.*, 2, 487 (1959).
5. G. Popják, J. W. Cornforth, R. H. Cornforth, R. Ryhage, and D. S. Goodman, *J. Biol. Chem.*, 237, 56 (1962).

## Carot-23
## Geranylgeranyl Pyrophosphate

**Formula:** $C_{20}H_{36}O_7P_2$
**Formula Wt.:** 450.40
**Calc. %:** C, 53.34; H, 8.04; O, 24.87; P, 13.75



**Sources:**

*Natural Sources.* Geranylgeranyl pyrophosphate does not normally accumulate in significant quantity in biological material. However, this compound is synthesized from mevalonate by a homogenate of the endosperm of immature, wild cucumber seeds.[1]

*Chemical Synthesis.* The synthesis of geranylgeranyl pyrophosphate by pyrophosphorylation of geranylgeraniol has been reported.[2]

**Methods of Purification:**

*Chromatography.* Geranylgeranyl pyrophosphate may be purified by countercurrent distribution between the two phases of 15:5:1:19 (v/v) butyl alcohol–isopropyl ether–ammonia–water.[2] Geranylgeranyl pyrophosphate may be further purified by chromatography on DEAE-cellulose. A linear gradient of 0.02 M potassium chloride in 1 mM tris buffer (pH 8.9) is used.[3]

**Methods of Assaying for Purity:**

*Phosphate Determination.* Geranylgeranyl pyrophosphate is treated[3] with 1 M HCl for 15 min at 100 °C. The liberated phosphate (2 mol/mol of geranylgeranyl pyrophosphate) is then determined by assay.[3]

*Chromatography.* Geranylgeranyl pyrophosphate may be assayed for purity by chromatography on DEAE-cellulose.[1,4] It may also be cleaved by treatment with 1 M HCl. The geranyllinalool and geranylgeraniol released are extracted into benzene, and then assayed by thin-layer chromatography on silica gel G plates in a solvent system of 9:1 (v/v) benzene–ethyl acetate.[3] Geranylgeranyl pyrophosphate may also be cleaved by treatment with bacterial alkaline phosphatase. The liberated geranylgeraniol is extracted into petroleum ether, and assayed by gas–liquid chromat graphy.[5]

*Enzymic Assay.* Geranylgeranyl pyrophosphate is enzymically converted into kaurene by a cell-free extract of *Echinocystis macrocarpa.*[3] Kaurene is extracted int acetone, and then assayed by thin-layer chromat graphy. Geranylgeranyl pyrophosphate is enzymically converted int phytoene by an enzyme system obtained from tomato fruit plastids.[6]

**Probable Impurities:** Geranylgeranyl phosphate and *cis*-isomers of geranylgeranyl pyr phosphate.

**Conditions of Storage:** Geranylgeranyl pyrophosphate is stored as a dry powder at low temperature (0 °C).

**References**


1. M. O. Oster and C. A. West, *Arch. Biochem. Biophys.*, 127, 112 (1968).
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3. B. B. Marsh, *Biochim. Biophys. Acta*, 32, 357 (1959).
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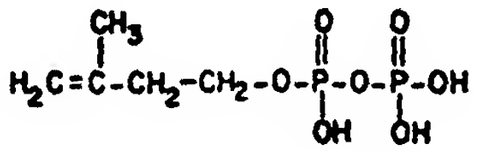
## Carot-24
## Isopentenyl Pyrophosphate
## (3-Methyl-3-buten-1-yl Pyrophosphate)

**Formula:** $C_5H_{12}O_7P_2$
**Formula Wt.:** 246.09
**Calc. %:** C, 24.41; H, 4.91; O, 45.51; P, 25.17



**Sources:** Synthesis of isopentenyl pyrophosphate is effected[1–8] through pyrophosphorylation of 3-methyl-3-buten-1-ol. Also, isopentenyl pyrophosphate may be synthesized enzymically from mevalonic acid.[4,6]

**Methods of Purification:**

*Derivative Formation.* Isopentenyl pyrophosphate is converted into the monocyclohexylammonium salt by passage through a column of Dowex-50 (cyclohexylammonium form) ion-exchange resin.[3] Isopentenyl pyrophosphate may also be converted into the lithium salt.[8]

*Ion-Exchange Chromatography.* Dowex-1 (formate form) is used to purify isopentenyl pyrophosphate.[4,6] Formic acid and ammonium formate are used as eluants. Isopentenyl pyrophosphate may also be purified by chromatography on a column of DEAE-cellulose.[6]

*Paper Chromatography.* Isopentenyl pyrophosphate has an $R_f$ value of 0.60 when chromatographed on paper (Whatman No. 1) in a system of 20:5:8 (v/v) *tert*-butyl alcohol–formic acid–water.[3] An $R_f$ value of 0.48 is found in 6:3:1 (v/v) 1-propanol–ammonia–water.[4]

**Methods of Assaying for Purity:**

*Phosphate Determination.* A standard assay may be used for the determination of phosphate.[3]

*Chromatography.* Isopentenyl pyrophosphate may be assayed for purity by paper[4] or ion-exchange chromatography.[4,5] The Rosenberg color reagent (for phosphate) is used to detect isopentenyl pyrophosphate on paper.[7]

*Enzymic Assay.* Radioactive isopentenyl pyrophosphate is converted into dimethylallyl pyrophosphate by isopentenyl pyrophosphate isomerase.[4] After acidification of the incubation mixture, this product is extracted into petroleum ether, and the extract is assayed for radioactivity. Isopentenyl pyrophosphate may also be cleaved by bacterial alkaline phosphatase or by snake-venom diesterase. The liberated alcohol may then be assayed by gas–liquid chromatography.[4]

*Melting Point.*[6] The tricyclohexylammonium salt melts at 145–147 °C.

*Infrared Spectrum.* The infrared spectrum of the cyclohexylammonium salt of isopentenyl pyrophosphate has been reported.[8]

**Conditions of Storage:** Store as the tricyclohexylammonium or the lithium salt.

**References**


1. F. Lynen, H. Eggerer, U. Henning, and I. Kessel, *Angew. Chem.* 70, 738 (1958).
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5. K. Bloch, S. Chaykin, A. H. Philips, and A. de Waard, *J. Biol. Chem.*, 234, 2595 (1959).
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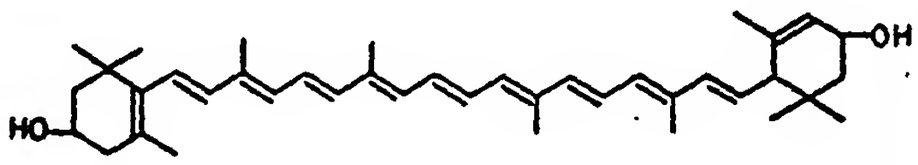
## Carot-25
## Lutein
## (3*R*,3′*S*,6′*R*)-β,ε-Carotene-3,3′-diol
## (Xanthophyll; α-Carotene-3,3′-diol)

**Formula:** $C_{40}H_{56}O_2$
**Formula Wt.:** 568.89
**Calc. %:** C, 84.45; H, 9.92; O, 5.63



**Sources:**

*Natural Sources.* Lutein is a major constituent of the xanthophyll fraction of many plants. It is present in appreciable proportions in green leaves,[1,2] red and yellow flowers (partly as the dipalmitate, helenien),[3] and in egg yolk.[4]

*Chemical Synthesis.* The synthesis of this compound has not yet been reported.

**Isolation Procedures:** The extraction, chromatography, and crystallization of lutein have been reported.[1–6]

**Methods of Purification:**

*Chromatography.* Lutein may be purified by chromatography on a column of magnesia or calcium hydroxide.[2,4,5]

*Crystallization.* A solvent pair frequently used for the crystallization of lutein is carbon disulfide–ethanol.[2,5]

*Derivative Formation.* The dipalmitate (helenien), other esters, ethers, and the ketone and perhydro derivatives, have been reported.[1,7]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of lutein may be determined by chromatography on magnesia or calcium hydroxide[2,6] or on kieselguhr paper.[3]

*Visible Spectrum.* Ethanol: 423, 446.5, and 477.5 nm. $E^{1\%}_{1cm}$ values 1750, 2560, and 2340, respectively. Spectral curve.[2]

*Mass Spectrum.* The mass spectrum of lutein has been reported.[9,10]

*Nuclear Magnetic Resonance*. The nuclear magnetic resonance spectrum of lutein has been reported.[11,12]

*Optical Rotatory Dispersion*. The optical rotatory dispersion curve of lutein has been reported.[12]

*Melting Point*.[3] Lutein melts at 151 °C.

*Optical Rotation*. $[\alpha]_{Cd}^{18}$ +160° (chloroform) has been reported.[3]

**Probable Impurities:** Oxidation products, *cis*-isomers, and possibly zeaxanthin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**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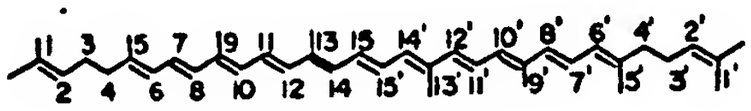
## Carot-26
## Lycopene
## ψ,ψ-Carotene

**Formula:** $C_{40}H_{56}$
**Formula Wt.:** 536.89
**Calc. %:** C, 89.48; H, 10.52




**Sources:**

*Natural Sources*. Tomato[1,2] and various other fruits and vegetables, animals,[3,4] and photosynthetic bacteria.[5]

*Chemical Synthesis*. The total synthesis of lycopene has been reported.[6–10]

**Isolation Procedures:** The isolation of lycopene has been described.[1–4,11,13]

**Methods of Purification:**

*Chromatography*. Column chromatography of the nonsaponifiable fraction is normally employed. The adsorbents most commonly used are deactivated alumina, calcium carbonate, calcium hydroxide, or magnesium oxide.[4,11]

*Crystallization*. Lycopene may be crystallized from the following solvent pairs:[3] carbon disulfide–methanol; ethyl ether–petroleum ether; acetone–petroleum ether. Lycopene is almost insoluble in methanol, moderately soluble in petroleum ether, benzene, or chloroform, and very soluble in carbon disulfide.

**Methods of Assaying for Purity:**

*Chromatography*. The purity of the compound may be determined by chromatography on circular filter paper having a suitable filler,[13,14] thin-layer chromatography,[15] or column chromatography.[1]



*Solvent Partition*.[16] The partition rati between petr leum ether and 95% methanol is 100:0.

*Visible Spectrum*. Petroleum ether (b.p. 40–60 °C): 446, 472, and 505 nm. $E_{1\,cm}^{1\%}$ 2250, 3450, and 3150, respectively. Spectral curve.[1,7,13] Benzene:[3] 455, 487, and 522 nm. Isomerization with iodine results in "*cis*-peak" absorption at 345 nm (minor peak) and 362 nm in petroleum ether.[17]

*Infrared Spectrum*. Infrared absorption spectra in chloroform and carbon disulfide have been reported.[7] The spectrum in a potassium bromide pellet has also been reported.[18]

*Nuclear Magnetic Resonance Spectrum*. The nuclear magnetic resonance spectrum has been reported.[19]

*Mass Spectrum*. The mass spectrum of lycopene has been reported.[20,21]

*Melting Point*. Lycopene melts at 172–173 °C in an evacuated tube.[7]

**Probable Impurities:** Oxidation products and *cis*-isomers; small proportions of related carotenes (neurosporene and hydroxylated carotenes) may also be present when lycopene is isolated from natural sources.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and a low temperature (−20 °C).

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17. L. Zechmeister, cis-trans *Isomeric Carotenoids, Vitamins A and Arylpolyenes*, Springer-Verlag, Vienna (1962).
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19. M. S. Barber, J. B. Davis, L. M. Jackman, and B. C. L. Weedon, *J. Chem. Soc.*, 2870 (1960).
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## Car t-27
## Lycoxanthin
## ψ,ψ-Caroten-16-ol
## (Lycopen-16-ol)

**Formula:** $C_{40}H_{56}O$
**Formula Wt.:** 552.90
**Calc. %:** C, 86.90; H, 10.21; O, 2.89

Lycoxanthin, previously thought to be lycopen-3-ol,[1] has recently been characterized as lycopen-16-ol.[2,3]

**Sources:**

*Natural Sources.* Lycoxanthin is found in fruits of *Solanum dulcamara*,[2-4] *Solanum esculentum*,[2,4,5] and *Tamus communis*.[4]

*Chemical Synthesis.* The chemical synthesis of lycoxanthin has been reported.[6]

**Isolation Procedures:** Lycoxanthin may be extracted from berries or fruit with ether, after dehydration of the tissues with ethanol.[4] The pigments of the extract are then transferred to benzene, and the lycoxanthin is purified by column chromatography.

**Methods of Purification:**

*Chromatography.* Lycoxanthin is purified by chromatography on a column of calcium carbonate, calcium hydroxide, deactivated alumina,[4,6] or alumina. The chromatogram is developed with benzene, and lycoxanthin is eluted with 3:1 benzene–methanol.[4]

*Crystallization.* Lycoxanthin can be crystallized from carbon disulfide or from a mixture of benzene and petroleum ether.[4]

**Methods of Assaying for Purity:**

*Visible Spectrum.*[4,7,8] Carbon disulfide: 473, 507, and 547 nm. Benzene:[4] 456, 487, and 521 nm. Petroleum ether:[7] 444, 472.5, and 503 nm. $E^{1\%}_{1cm}$ 3360 at 472.5 nm.

*Infrared Spectrum.* The infrared spectra of lycoxanthin and some of its derivatives have been reported.[2,8]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectra of lycoxanthin and lycoxanthin acetate have been reported.[2,8]

*Mass Spectrum.* The mass spectrum of lycoxanthin has been reported.[9]

*Melting Point.* Lycoxanthin has been reported to melt at 168 °C,[8] and, after crystallization from ethyl ether–light petroleum, at 173–174 °C (uncorr.)[2,6]

*Derivatives.* The acetate of lycoxanthin has been prepared.[4] This compound crystallizes from benzene–methanol and it has a melting point of 137 °C.[4] The aldehyde derivative of lycoxanthin has also been prepared.[8]

**Probable Impurities:** Lycophyll and oxidation products.

**Conditions of Storage:** Darkness (brown vial), an inert atmosphere (sealed ampoule), and cold temperature (−20 °C).

**References**


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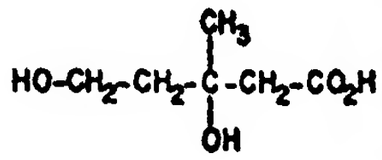
8. P. Karrer and E. Jucker, *Carotenoids*, E. A. Braude (translator), Elsevier, New York (1950).
9. C. R. Enzell, G. W. Francis and S. Liaaen-Jensen, *Acta Chem. Scand.*, 23, 727 (1969).


## Carot-28
## Mevalonic Acid
## (3,5-Dihydroxy-3-methylpentanoic Acid)

**Formula:** $C_6H_{12}O_4$
**Formula Wt.:** 148.16
**Calc. %:** C, 48.63; H, 8.17; O, 43.20



**Sources:** Prepared synthetically from 4-acetoxy-2-butanone and ethyl bromoacetate.[1,2]

**Methods of Purification:**

*Derivative Formation.* Synthesis yields a racemic mixture of ethyl 5-acetoxy-3-hydroxy-3-methylpentanoate.[2] This product is hydrolyzed to a mixture of (±)-mevalonic acid and (±)-mevalono-1,5-lactone. The lactone may then be isolated by short-path distillation, and crystallized from acetone–ether.[1] It may also be converted into a benzhydrylamide derivative.[3] The *N,N'*-dibenzylethylenediammonium (DBED) salt of mevalonic acid may also be prepared and crystallized.[1,3] Only the natural (+) optical isomer of mevalonic acid is biologically active.[4] The biologically active isomer of mevalonolactone has the (*R*)-(−) configuration.[5]

**Methods of Assaying for Purity:**

*Bioassay.* Mevalonic acid and mevalonolactone may be determined quantitatively by microbiological assay with *Lactobacillus acidophilus* ATCC 4963.[6]

*Enzymic Assay.* Mevalonic acid may be determined quantitatively by spectrophotometric assay in a system in which mevalonic acid is converted into phosphomevalonic acid with mevalonic acid kinase.[7]

*Hydroxamate Assay.* Mevalonolactone may be converted into the hydroxamate, and the quantity of this compound may be determined spectrophotometrically.[8]

*Chromatography.* Mevalonic acid may be chromatographed on paper or on a Dowex-1 (formate) column.[9] These methods are particularly useful if the mevalonic acid is radioactive. Mevalonic acid may also be assayed (as the lactone) by gas–liquid chromatography.[10]

*Melting Point.* Mevalonolactone[3] 27–28 °C; DBED salt[3] 124–125 °C; benzhydrylamide derivative[3] 93–95 °C.

*Infrared Spectra.* Strong bands are observed at 2.90–2.95 μm (hydroxyl function) and at 5.78 μm (ester function), for mevalonolactone in chloroform.[3]

**Conditions of Storage:** As the DBED salt, or as the lactone, in a sealed container in a refrigerator.

**References**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9. K. Bloch, S. Chaykin, A. H. Philips, and A. de Waard, *J. Biol. Chem.*, 234, 2595 (1959).
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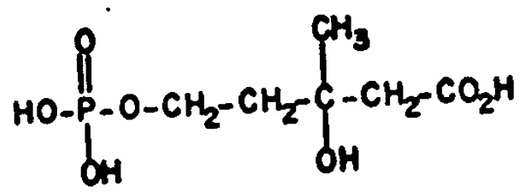
## Car t-29
## Mevalonic Acid 5-Phosphate
## (3,5-Dihydroxy-3-methylpentanoic Acid 5-Phosphate)

**Formula:** $C_6H_{13}O_7P$
**Formula Wt.:** 228.14
**Calc. %:** C, 31.59; H, 5.72; O, 49.09; P, 13.58



**Sources:** Prepared synthetically by phosphorylation of mevalonic benzhydrylamide[1] or reduced mevalonolactone, followed by oxidation.[2] Mevalonic acid 5-phosphate may also be synthesized enzymically from mevalonic acid.[3,4] Only the natural (*R*) optical isomer of mevalonic acid is converted into the phosphate.

**Methods of Purification:**

*Derivative Formation.* Mevalonic acid phosphate is converted into the tricyclohexylammonium salt by treatment with cyclohexylamine. The salt is then crystallized from water–acetone at −15 °C.[2]

*Chromatography.* Mevalonic acid phosphate may be purified by ion-exchange chromatography.[1,5] Purification may also be effected by paper chromatography (Whatman No. 1) in a system of isobutyric acid–ammonia–water (66:3:30 v/v).[2] An $R_f$ of 0.42 is obtained. The presence of phosphate at this $R_f$ may be shown by spraying with Rosenberg's reagent.[6]

**Methods of Assaying for Purity:**

*Phosphate Determination.* A standard assay may be used for the determination of phosphate.[2,5]

*Enzymic Assay.* Mevalonic acid phosphate may be assayed enzymically through conversion into mevalonic acid pyrophosphate by phosphomevalonic kinase. A spectrophotometric method has been developed for this assay.[3,4]

*Chromatography.* The purity of mevalonic acid phosphate may be determined by paper chromatography.[2]

*Melting Point.* Melting points of 145–147 °C for the cyclohexylammonium salt[7] and 154–156 °C for the tricyclohexylammonium salt[8] have been reported.

**Conditions of Storage:** Store as the cyclohexylammonium salt.

**References**

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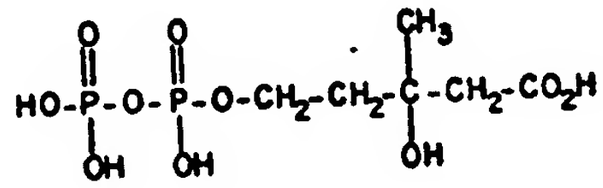
## Carot-30
## Mevalonic Acid 5-Pyrophosphate
## (3,5-Dihydroxy-3-methylpentanoic Acid 5-Pyrophosphate)

**Formula:** $C_6H_{14}O_{10}P_2$
**Formula Wt.:** 308.13
**Calc. %:** C, 23.38; H, 4.58; O, 51.93; P, 20.11



**Sources:**

*Natural Sources.* Mevalonic acid 5-pyrophosphate does not normally accumulate in plant or animal tissue. However, this compound can be synthesized in small amounts by phosphomevalonic kinase. The synthesis of this compound from mevalonic acid or mevalonic acid 5-phosphate by partially purified enzymes from yeast,[1,2] pig liver,[3] and rat liver[4] has been reported.

*Chemical Synthesis.* The chemical synthesis of mevalonic acid 5-pyrophosphate has not yet been reported.

**Isolation Procedures:**[4,6] The incubation mixture is deproteinized with acid, and the precipitated protein is washed thoroughly with water. The supernatant solution is then subjected to chromatography.

**Methods of Purification:**

*Chromatography.* Mevalonic acid 5-pyrophosphate may be purified by ion-exchange chromatography on Dowex-1 formate[1] or DEAE-cellulose[5–7] or by paper chromatography.[1–4,6]

**Methods of Assaying for Purity:**

*Chromatography.* The foregoing methods may be used to assay for the purity of a preparation of mevalonic acid 5-pyrophosphate.

*Chemical Methods.* Mevalonic acid 5-pyrophosphate may be cleaved with alkaline phosphatase.[8] Assays may then be made for mevalonic acid by gas–liquid chromatography[9] or for phosphate.[10] Quantitative assays for these compounds may be performed.[9,10]

**Probable Impurities:** Adenosine triphosphate and mevalonic phosphate.

**Conditions of Storage:** As a dry powder, or in a slightly alkaline, aqueous solution (pH 7–9) at a low temperature (−20 °C).

**References**

1. W. Henning, E. M. Möslein, and F. Lynen, *Arch. Biochem. Biophys.*, 83, 259 (1959).
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## Carot-31
## Nerolidol
## (3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol)

**Formula:** $C_{15}H_{26}O$
**Formula Wt.:** 222.37
**Calc. %:** C, 81.02; H, 11.78; O, 7.20


**Sources:**

*Natural Sources.* Nerolidol is obtained from cajaput,[1] camphor,[2] grapefruit,[3] lime,[4] neroli,[5] and coriander fruit oils,[6] orange blossoms,[7] and Peru balsam.[7] Nerolidol is obtained from these oils by fractional distillation.

*Chemical Synthesis.* Nerolidol has been synthesized through the condensation of linalool with diketene, and subsequent hydrolysis and reduction of the intermediate. A 96% yield of pure product has been reported.[8] It has also been synthesized by condensation of cyclopropyl methyl ketone with the magnesium derivative of 1-bromo-4,8-dimethyl-3,7-nonadiene at 75 °C. A 20% yield of product was reported.[9]

**Methods of Purification:**

*Chromatography.* Nerolidol may be purified[10] by thin-layer chromatography on plates of kieselguhr G. It may also be purified by thin-layer chromatography on a plate of silica gel that has been impregnated with increasing concentrations of silver nitrate. 1,2-Dichloroethane–chloroform–ethyl acetate–propyl alcohol (10:10:1:1) is used as the solvent system.[11] Thin-layer plates (26 × 76 mm) of silica gel, 250 μm thick, may also be used, with 4:1 ethyl acetate–hexane.[12] Separation and purification of nerolidol has been effected[13,14] by gas–liquid chromatography on butanediol succinate (20%) on Chromosorb W.

**Methods of Assaying for Purity:**

*Chromatography.* The foregoing techniques of thin-layer and gas–liquid chromatography may be used to assay for the purity of nerolidol.

*Ultraviolet Spectrum.* The ultraviolet absorption spectrum of nerolidol in cyclohexane shows a maximum at 187–192 nm.[14,15]

*Infrared Spectrum.* The infrared spectrum of nerolidol has been reported.[17]

*Refractive Index.* The $n_D^{20}$ for the isomers of nerolidol are[18] *dextro*, 1.4898; *levo*, 1.4799; DL, 1.4801.

*Specific Rotation.* The $[\alpha]_D^{20}$ for the isomers of nerolidol are *dextro*,[18] +142°; *levo*,[18] −6.5°; DL,[7] +15.5°.

*Density.* The density of nerolidol at 20 °C is 0.8778 g/ml.[7]

*Solubility.* Nerolidol is soluble in alcohol, ether, and other organic solvents.[18]

*Derivatives.* The phenylurethan (m.p. 37–38 °C, b.p. 145–146 °C), semicarbazone (m.p. 134–135 °C) and acetate (b.p. 128–129 °C at 1.6 mmHg) derivatives of nerolidol have been reported.[7]

*Boiling Point.*[7] Nerolidol has been reported to boil at 276 °C.

*Probable Impurities. cis*-Isomers and farnesol.

*Conditions of Storage.* Nerolidol should be stored in tightly sealed containers, protected from light, in a cool place.

**References**


1. V. K. Sood, *Perfum. Essent. Oil Rec.*, 57, 362 (1966); *Chem. Abstr.*, 65, 8661g (1966).
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## Carot-32
## Neurosporene
## 7,8-Dihydro-ψ,ψ-carotene

**Formula:** $C_{40}H_{58}$
**Formula Wt.:** 538.91
**Calc. %:** C, 89.15; H, 10.85

**Sources:**

*Natural Sources.* Neurosporene is widely distributed in small amounts in fungi,[1,2] fruits and vegetables,[3,4] and photosynthetic bacteria.[5]

*Chemical Synthesis.* The total synthesis of neurosporene has been reported.[6,7]

**Isolation Procedures:** Neurosporene is extracted from natural sources[1,3] with a solvent such as acetone, ethanol, or methanol. The extract is then transferred to petroleum ether, with or without saponification, and subjected to column chromatography.

**Methods of Purification:**

*Chromatography.* Neurosporene is purified by chromatography on 1:1 magnesium oxide–Celite,[3] calcium hydroxide–Celite,[3] or alumina.[8]

**Methods of Assaying for Purity:**

*Chromatography.* Assays for the purity of neurosporene may be conducted by chromatography on calcium hydroxide–Celite,[3] magnesium oxide–Celite,[3] or alumina.[8] Thin-layer chromatography may also be performed.[9]

*Visible Spectrum.* Petroleum ether:[9] 416, 440, and 470 nm. $E_{1\,cm}^{1\%}$ 2990 at 440 nm. Hexane:[3] 415, 438.5, and 469 nm. $E_{1\,cm}^{1\%}$ 1920, 2990, and 3010, respectively. Carbon disulfide:[3] 439.5, 470.5, and 502.5 nm.

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of neurosporene has been reported.[6,7]

*Mass Spectrum.* The mass spectrum of neurosporene has been reported.[10,11]

*Melting Point.* A melting point of 124 °C has been reported.[2]

**Probable Impurities:** Oxidation products, *cis*-isomers, γ-carotene, and *cis*-isomers of lycopene.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

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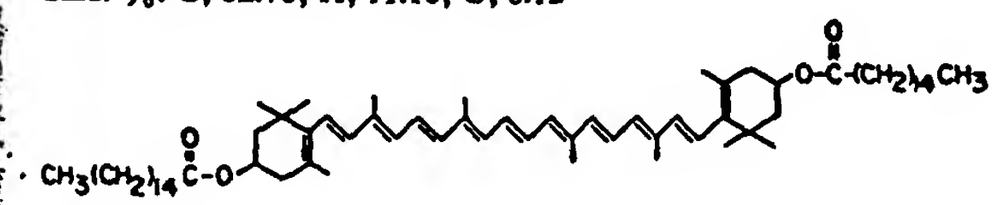
## Carot-33
## Physalien
## (3*R*,3′*R*)β,β-Carotene-3,3′-diol Dipalmitate
## (Zeaxanthin Dipalmitate)

**Formula:** $C_{72}H_{116}O_4$
**Formula Wt.:** 1045.73
**Calc. %:** C, 82.70; H, 11.18; O, 6.12




**Sources:**

*Natural Sources.* Physalien occurs in a wide variety of plant materials. It was first isolated from the sepals of *Physalis alkekengi* and *Physalis franchetii*.[1] Since then, it has been reported to be present in *Lycium halimifolium*,[2] *Lycium barbarum*,[2] *Solanum hendersonii*,[3] *Aspargus officinalis*,[3] and *Hippophaes rhamnoides*.[3,4]

*Chemical Synthesis.* Zeaxanthin dipalmitate has been synthesized from zeaxanthin.[5] The total synthesis of zeaxanthin dipalmitate has also been reported.[6]

**Isolation Procedures:**[3] The sepals of *Physalis alkekengi* are exhaustively extracted with benzene. The combined extracts are concentrated to a small volume, and the pigment is precipitated or crystallized by the addition of ethanol[3] or acetone.[5] The pigment may also be purified by column chromatography.

**Methods of Purification:**

*Chromatography.* Physalien may be purified by chromatography on water-deactivated aluminum oxide. Hexane–ethyl ether (19:1) is used to develop the column.

*Crystallization.*[7] Physalien has been crystallized from benzene–methanol and from petroleum ether–ethanol.

**Methods of Assaying for Purity:**

*Chromatography.* The purity of physalien can be determined by chromatography on a column of aluminum oxide or magnesium oxide. Chromatograms are developed with chloroform or 19:1 hexane–ethyl ether when aluminum oxide is used and with dichloromethane when magnesium oxide is the adsorbent.

*Visible Spectrum.* Hexane: 449 and 478 nm. $E^{1\%}_{1\,cm}$ 1410 and 1255, respectively. Petroleum ether: 452 and 480 nm. $E^{1\%}_{1\,cm}$ 1335 and 1190, respectively.[6] Cyclohexane: 454 and 483 nm. $E^{1\%}_{1\,cm}$ 1350 and 1180, respectively. Benzene: 463 and 492 nm. $E^{1\%}_{1\,cm}$ 1250 and 1090, respectively.

*Melting Point.* Physalien has been reported to melt at 95–96 °C (corr., under vacuum),[5] and at 98.5–99.5 °C.[7]

**Probable Impurities:** Oxidation products, *cis*-isomers, and, possibly, zeaxanthin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and a low temperature (0 °C).

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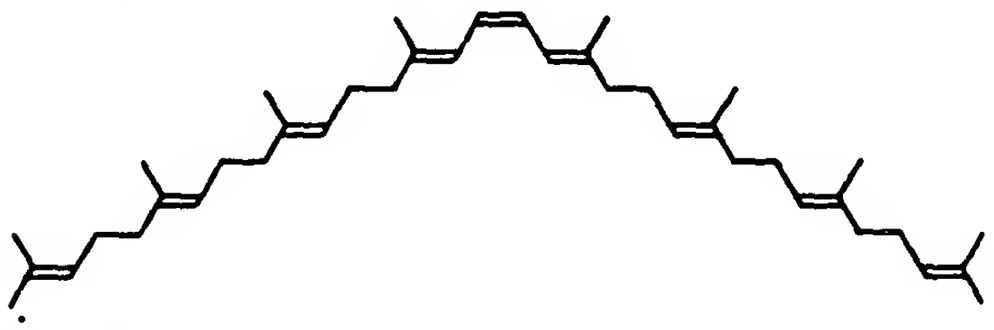
## Carot-34
## Phytoene
## 15-*cis*-7,8,11,12,7′,8′,11′,12′-Octahydro-ψ,ψ-carotene
## (7,8,7′,8′,11,12,11′,12′-Octahydrolycopene)

**Formula:** $C_{40}H_{64}$
**Formula Wt.:** 544.96
**Calc. %:** C, 88.16; H, 11.84



**Sources:**

*Natural Sources.* Phytoene is rather widely distributed in carotenoid-containing fruits and some other tissues not containing chlorophyll. The best sources are tomato fruits,[1–4] tomato paste,[5] and carrot oil.[5] The naturally occurring isomer has a central *cis*-configuration.[6]

*Chemical Synthesis.* Phytoene has been synthesized from all-*trans*-geranyllinalool.[7,8]

**Isolation Procedures:** Phytoene is extracted from plant materials with a solvent such as acetone, ethanol, or methanol, and then transferred into petroleum ether.[1–5] This compound is then purified by chromatography, with or without prior treatment with alcoholic potassium hydroxide.

**Methods of Purification:**

*Chromatography.* Phytoene is purified by chromatography on a column of magnesia–Supercel,[5] or alumina.[5,6]

**Methods of Assaying for Purity:**

*Chromatography.* Phytoene may be assayed for purity by chromatography on magnesia–Supercel,[5] or alumina.[5,6] It may also be assayed for purity by thin-layer chromatography on silica gel.[5]

*Ultraviolet Spectrum.* Hexane: 275, 285, and 297 nm.[6] $E^{1\%}_{1\,cm}$ 850 (286 nm).[6] The ultraviolet spectrum of all-*trans*-phytoene has also been reported.[6]

*Infrared Spectrum.* The infrared spectrum of central-*cis*-phytoene and all-*trans*-phytoene have been reported.[5,6,10]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum f phytoene has been reported.[6,7,11]

*Mass Spectrum.* The mass spectrum of phytoene has been reported.[12,13]

*Melting Point.* Phytoene has not yet been crystallized. On cooling it forms a colorless glassy mass.

**Probable Impurities:** Waxes and *cis–trans*-isomers formed during isolation.

**Conditions of Storage:** In solution in petroleum ether under nitrogen at a low temperature (−20 °C).

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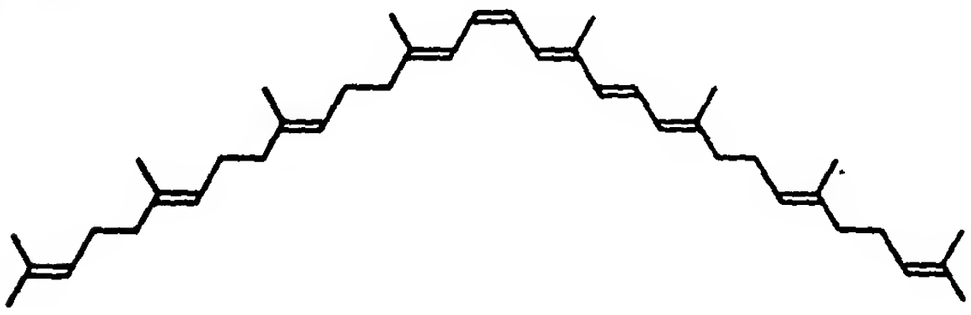
## Carot-35
## Phytofluene
## 15-*cis*-7,8,11,12,7′,8′-Hexahydro-ψ,ψ-carotene
## (7,8,7′,8′, 11′,12′-Hexahydrolycopene)

**Formula:** $C_{40}H_{62}$
**Formula Wt.:** 542.94
**Calc. %:** C, 88.49; H, 11.51



**Sources:**

*Natural Sources.* Phytofluene is rather widely distributed in carotenoid-containing fruit and in some other tissues not containing chlorophyll. It is found in persimmons,[1] red peppers,[1] carrots,[2] tomato fruits,[3,4] and tomato paste.[5,6] all-*trans*-Phytofluene also occurs naturally.[7]

*Chemical Synthesis.* Phytofluene has been synthesized chemically.[8,9]

**Isolation Procedures:** Phytofluene is extracted from plant materials with such solvents as acetone, ethanol, and methanol and is then transferred into petroleum ether.[1–6] It is then purified by chromatography, with or without a prior treatment with alcoholic potassium hydroxide.

**Methods of Purification:**

*Chromatography.* Phytofluene is purified by chromatography n calcium hydroxide–alumina,[5,6] magnesium oxide–Supercel,[10] or alumina.[11] all-*trans*-Phytofluene is readily separated from *cis*-phytofluene on partially deactivated alumina.[7]

**Methods of Assaying for Purity:**

*Chromatography.* Phytofluene may be assayed for purity by chromatography on calcium hydroxide–alumina,[5,3] magnesium oxide–Supercel,[10] or alumina.[11] Assay may also be made by chromatography on paper[13] or on a thin layer of silica gel.[13] The position of phytofluene on a chromatographic column may be readily determined by its characteristic green–white fluorescence when exposed to ultraviolet light of long wavelength.

*Ultraviolet Spectrum.* Petroleum ether:[3,6,14] 331, 348, and 367 nm. $E^{1\%}_{1\,cm}$ 1350 at 348 nm. Benzene:[15] 338, 355, and 374 nm. The ultraviolet spectrum of all-*trans*-phytofluene has been reported.[6,15]

*Infrared Spectrum.* The infrared spectrum of phytofluene has been reported.[8,16]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of phytofluene has been reported.[8,17]

*Mass Spectrum.* The mass spectrum of phytofluene has been published.[18,19]

*Melting Point.* Phytofluene has not been crystallized. On cooling, phytofluene forms a glassy mass lacking crystalline structure.

**Probable Impurities:** Phytoene, *trans*-phytofluene, and oxidati n products.

**Conditions of Storage:** In solution in petroleum ether under nitrogen at a low temperature (−20 °C).

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## Carot-36
## Prolycopene

Formula: $C_{40}H_{56}$
Formula Wt.: 536.90
Calc. %: C, 89.49; H, 10.51

Prolycopene is a poly-*cis*-lycopene of unknown stereochemical configuration. However, the suggestion has been made that it may be a symmetrical penta-*cis*-lycopene containing a central *cis*- and four other, unhindered, *cis*-double bonds.[1]

Sources:

*Natural Sources.* Prolycopene is found in small amounts in various fruits and flowers.[2] However, the best source of this pigment is the ripe fruit of the "tangerine" or "golden jubilee" type of tomato.[3–6]

*Chemical Synthesis.* The chemical synthesis of prolycopene has not yet been reported.

Isolation Procedures:[3,4] Plant tissue is extracted with a solvent such as acetone, methanol, or ethanol and the carotenes are then transferred into petroleum ether. Prolycopene in this extract is then purified by chromatography, either with or without prior saponification with alcoholic potassium hydroxide.

Methods of Purification:

*Chromatography.* Prolycopene may be purified by chromatography on calcium hydroxide,[3] magnesium oxide–Supercel,[5] or deactivated alumina.[6]

*Crystallization.* Prolycopene has been crystallized from petroleum ether.[3]

Methods of Assaying for Purity:

*Chromatography.* The purity of prolycopene may be determined by chromatography on calcium hydroxide,[3,7] magnesium oxide–Supercel,[5] or deactivated alumina.[6]

*Visible Spectrum.* Petroleum ether: 443.5 and 470 nm.[1] $E^{1\%}_{1cm}$ 1920.[8] Carbon disulfide: 469.5 and 500.5 nm.[8] Benzene: 454.5 and 485 nm.[2] Chloroform: 453.5 and 484 nm.[8]

*Infrared Spectrum.* The infrared spectrum of prolycopene has been reported.[9]

*Melting Point.*[3] Prolycopene melts at 111 °C.

Probable Impurities: Oxidation products, and other isomers of lycopene.[10]

Conditions of Storage: Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**


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## Carot-37
## Proneurosporene
## (Synonyms: Protetrahydrolycopene, Neoneurosporene P, Unidentified Carotene I, and Poly-*cis*-ψ-carotene)[1]

Formula: $C_{40}H_{58}$
Formula Wt.: 538.91
Calc. %: C, 89.15; H, 10.85

Proneurosporene is a poly-*cis*-neurosporene that contains a *trans*-double bond at the middle of the molecule.[2]

Sources:

*Natural Sources.* The principal sources of proneurosporene are the ripe berries of *Pyracantha angustifolia*[2] and fruits of the "golden jubilee" and "tangerine" varieties of tomato.[3]

*Chemical Synthesis.* The chemical synthesis of proneurosporene has not yet been reported.

Isolation Procedures: Berries or fruit are extracted with a solvent such as acetone, ethanol, or methanol. The carotenes are then transferred into petroleum ether and purified by chromatography, with or without prior saponification with alcoholic potassium hydroxide.

Methods of Purification:

*Chromatography.* Proneurosporene is purified by chromatography on 2:1 calcium hydroxide–Celite,[2] 3:1:1 magnesium oxide–calcium hydroxide–Celite,[3] 1:1 magnesium oxide–Supercel,[3] or deactivated alumina.[4]

Methods of Assaying for Purity:

*Chromatography.* Assays for the purity of proneurosporene may be carried out by chromatography on columns of the adsorbents just given.[3–4]

*Visible Spectrum.*[3] Hexane: 408, 432, and 461 nm. $E^{1\%}_{1cm}$ 2040 at 432 nm.

*Infrared Spectrum.* The infrared spectrum of proneurosporene has been reported.[5]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of proneurosporene has not yet been reported.

*Melting Point.* Proneurosporene has not yet been crystallized.

Probable Impurities: Prolycopene, other *cis*-isomers of neurosporene, and oxidation products.

Conditions of Storage: Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C).

**References**


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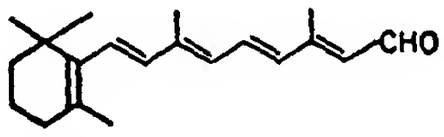
3. H. H. Trombly and J. W. Porter, *Arch. Biochem. Biophys.*, 43, 443 (1953).
4. S. C. Kushwaha, G. Suzue, C. Subbarayan, and J. W. Porter, *J. Biol. Chem.*, 245, 4708 (1970).
5. K. Lunde and L. Zechmeister, *J. Am. Chem. Soc.*, 77, 1647 (1955).

## Carot-38
## Retinal
## (Vitamin $A_1$ Aldehyde)

**Formula:** $C_{20}H_{28}O$
**Formula Wt.:** 284.44
**Calc. %:** C, 84.45; H, 9.92; O, 5.63




**Isomers:** Six isomers of retinal have been reported:[1] all-*trans*; 13-*cis* (neo-a); 11-*cis* (neo-b); 9-*cis* (iso-a); 9,13-di-*cis* (iso-b); and 11,13-di-*cis* (neo-c).

**Bipotency:** All-*trans*-retinal and 13-*cis*-retinal have 91% of the biological activity of all-*trans*-retinyl acetate. Other isomers (*cis*) of retinal have a lower biological activity.[2]

**Sources:**

*Natural Sources.* All-*trans*-retinal is present in herring roe and in hens' eggs.[3] In the eyes of animals, marine fish, and crustacea, retinal is present as the 11-*cis* isomer.[4]

*Chemical Synthesis.* Retinal is formed from retinol by oxidation with activated $MnO_2$ in petroleum ether.[5]

**Methods of Purification:** Retinal may be separated from retinol and its esters by column chromatography. Retinal and its isomers may then be crystallized from petroleum ether; or their semicarbazones from ethanol; or their (2,4-dinitrophenyl)hydrazones from ethyl acetate.[6]

**Methods of Assaying for Purity:**

*Column and Thin-Layer Chromatography.* The adsorbents used for the chromatography of retinal are similar, or identical, to those used for the chromatography of retinol and its derivatives.[7,8] Retinal is eluted from water-deactivated alumina columns with 1–2% acetone. When retinal is chromatographed on thin-layer plates of silica gel G, the chromatograms are developed with ether–hexane (1:1). The isomers of retinal are partially separated in each of the above systems.

*Gas–Liquid Chromatography.* Retinal is more stable than retinol or retinyl acetate on gas–liquid columns. It can be recovered quantitatively under proper conditions.[9] However, isomerization occurs at high temperatures.

*Ultraviolet Spectrum.* The $E^{1\%}_{1\,cm}$ values and the absorption maxima of retinal and its isomers in ethanol have been reported as follows:[1,8,10] all-*trans*, 1530 (381 nm); 13-*cis*, 1250 (375 nm); 11-*cis*, 878 (376 nm); 9-*cis*, 1270 (373 nm); 9,13-di-*cis*, 1140 (368 nm); and 11,13-di-*cis*, 700 (373 nm).

*Other Spectra.* The infrared[6] and fluorescence[11] spectra of retinal have been reported.

*Melting Points.* The melting points of retinal and its isomers have been reported as follows:[10] all-*trans*, 57 and 65 °C; 13-*cis*, 77 °C; 11-*cis*, 64 °C; 9-*cis*, 64 °C; and 9,13-di-*cis*, 49 and 85 °C.

**Quantitative Assays:** The quantity of retinal is most frequently determined by ultraviolet absorption spectroscopy.[1,8,10] However, retinal forms a transient, highly colored complex with antimony trichloride,[3] trifluoroacetic acid,[12] and other Lewis acids. Hence, the quantity of retinal may also be determined through measurement of the quantity of light absorbed by this complex. The highest recent value of $E^{1\%}_{1\,cm}$ at 666 nm is 4150.[3] Previous values have been as low as 3340.[8,13] The presence of acetic anhydride affects the absorption maximum and the extinction coefficient. Various retinal isomers, but not retinol, also react with thiobarbituric acid. The complex has $E^{1\%}_{1\,cm}$ 2040 at 530 nm.[13]

The 9-*cis*, 11-*cis*, and 9,13-di-*cis* isomers of retinal react with opsin (isolated from the retina) to yield rhodopsin or isorhodopsin.[14,15] In 2% digitonin solution, the $E^{1\%}_{1\,cm}$ values for the opsin complexes are 9-*cis*, 1439 (487 nm); 11-*cis*, 1467 (500 nm); and 9,13-di-*cis*, 1271 (487 nm). The retinol isomers having 11-*cis* or 13-*cis* double bonds may be distinguished by the maleic anhydride test.[8,16]

**Probable Impurities:** Retinol, *cis*-isomers, and oxidation products.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C). Solutions of retinal in pure solvents are reasonably stable in the dark at low temperatures.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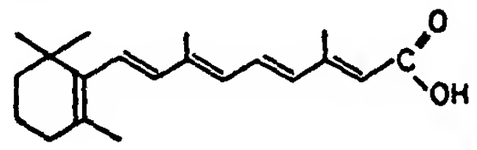
## Carot-39
## Retinoic Acid
## (Vitamin $A_1$ Acid)

**Formula:** $C_{20}H_{28}O_2$
**Formula Wt.:** 300.44
**Calc. %:** C, 79.96; H, 9.39; O, 10.65




**Isomers:** The four unhindered isomers of retinoic acid (all-*trans*, 9-*cis*, 13-*cis*, and 9,13-di-*cis*) have been crystallized and characterized.[1]

**Biopotency:** All-*trans*-retinoic acid has 10–141% of the growth-promoting activity of retinol. This discrepancy in activity is attributable to the method of administration of retinoic acid to the animal. *cis*-Isomers of retinoic acid have less biological activity than the all-*trans*-compound. Retinoic acid does not fulfill the visual or reproductive functions of retinol or retinal.[2]

**Sources:**

*Natural Sources.* Traces of retinoic acid are found in liver and bile after the administration of retinal;[3] larger amounts are excreted in the bile as the β-D-glucosiduronic acid.[4]

*Chemical Synthesis.* Retinoic acid is prepared from retinal by oxidation with silver oxide, or as an intermediate in the synthesis of retinol.[1]

**Methods of Purification:** Retinoic acid may be separated from many other compounds by chromatography on columns of silicic acid. Complete purification of the compound is achieved by crystallizati n from methanol, ethanol, or isopropyl alcohol. Methyl retinoate is crystallized from methanol.[5]

**Methods of Assaying for Purity:**

*Column and Thin-Layer Chromatography.* Retinoic acid is eluted from silicic acid columns by small proportions of ethanol in hexane. This compound also migrates well on a thin-layer plate of silica gel G when a solvent system of 4:1:1 benzene–chloroform–methanol[6] is used. Retinyl β-D-glucosiduronic acid also migrates on silica gel G plates in 5:5:5:1 benzene–chloroform–methanol–acetic acid.[4]

*Partition Chromatography.* Retinoic acid may be separated from retinol on silicone-treated paper (reverse phase) when various polar solvents are used to develop the chromatogram.[7]

*Ion-Exchange Chromatography.* Retinoic acid is eluted from a DEAE-cellulose column by 0.04 M HCl in ethanol,[7] and from a Biorad AG-2-X8 anion-exchange column with 5:95 acetic acid–methanol.[8]

*Gas–Liquid Chromatography.* Methyl retinoate may be assayed for purity by gas–liquid chromatography. This compound may also be recovered quantitatively, since it is quite stable at elevated temperatures.[8]

*Ultraviolet Spectrum.* The $E^{1\%}_{1cm}$ values of retinoic acid and its isomers[9–11] in purified ethanol[5] are: all-*trans*, 1500 (350 nm); 13-*cis*, 1320 (354 nm); 9-*cis*, 1230 (345 nm); and 9,13-di-*cis*, 1150 (346 nm).

*Melting Point.* Melting points for retinoic acid and its isomers have been reported as follows: all-*trans*, 180 °C; 13-*cis*, 175 °C; 9-*cis*, 191 °C; and 9,13-di-*cis*, 136 °C.[1,10]

**Quantitative Assays:** Ultraviolet absorption spectroscopy is most commonly used to determine the quantity of retinoic acid.[9–11] The amount of light absorbed by the transient highly colored complex of retinoic acid with antimony trichloride,[12] trifluoroacetic acid,[13] and other Lewis acids is also a measure of the quantity of retinoic acid. An $E^{1\%}_{1cm}$ value of 1770 (574 nm) has been reported for this complex.[13]

**Probable Impurities:** *cis*-Isomers and oxidation products.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C). Solutions of the acid in pure organic solvents in the dark are reasonably stable, whereas aqueous solutions of the acid deteriorate rapidly.

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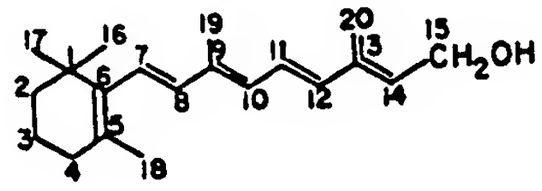
# Carot-40
# Retinol
# (Vitamin $A_1$ Alcohol)

**Formula:** $C_{20}H_{30}O$
**Formula Wt.:** 286.46
**Calc. %:** C, 83.86; H, 10.56; O, 5.59




**Nomenclature:** "Vitamin $A_1$ alcohol" has been designated "retin 1" by the Commission on Nomenclature of Biological Chemistry,[1] and the stereochemistry of methyl groups at C-1 has been assigned by analogy with that of lanostane.

**Isomers:** Six isomers of vitamin A have been reported: all-*trans*; 13-*cis* (neo-a); 11-*cis* (neo-b); 9-*cis* (iso-a); 9,13-di-*cis* (iso-b); and 11,13-di-*cis* (neo-c).[2]

**Biopotency:** Pure all-*trans*-retinol has $3.333 \times 10^6$ I.U./g. Both the U.S.P. (United States Pharmacopeia) unit and International Unit (I.U.) are defined as the amount of all-*trans*-retinyl acetate (0.344 μg) having the biological activity of 0.300 μg of all-*trans*-retinol.

**Sources:**

*Natural Sources.* The best sources are liver oils of marine fish, where vitamin $A_1$ occurs mainly as retinyl esters. Free retinol is also present in the blood and tissues of vertebrates and in the eyes of crustacea.

*Chemical Synthesis.* Many procedures have been reported for the synthesis of retinol. These include synthesis from acetone and acetylene,[3] from β-ionone via condensation with methyl 3-methylglutaconate,[4] from β-ionone via vinyl-β-ionol by the Wittig reaction,[5] and from β-ionone via a $C_{14}$-aldehyde (Darzens' reacti n) followed by Grignard addition of 3-methyl-2-penten-4-yn-1-ol.[6] These methods of synthesis have been reviewed.[6–8]

**Methods of Purification:**

*Crystallization.* In the past, retinol or its esters were isolated from fish-liver oils by molecular distillation. At present, however, high-potency concentrates and crystalline retinol, or its derivatives, are generally prepared by chemical synthesis. Solvated crystals of all-*trans*-retinol are obtained from methanol or ethyl formate. Solvent-free crystals are obtained from propylene oxide r petroleum ether.[9,10]

*Column Chromatography.* Many adsorbents have been employed, including alumina, dicalcium phosphate, calcium carbonate, magnesium oxide, magnesium carbonate, silicic acid, and bone meal.[11] Columns of water-deactivated alumina are c mmonly used; from these, retinol is eluted quantitatively with 3–5% acetone in hexane. Isomers of retinol may be separated n columns of dicalcium phosphate[12,13] or on thin-layer plates of silica gel G developed with petroleum ether (low boiling)—methyl-heptenone (11:2).[14] Thin-layer plates of water-deactivated alumina have also been used with various solvents.[15] Retinol may be detected by fluorescence under ultraviolet light, or by reaction with iodine vapor.

*Partition Chromatography.* Various adsorbents, impregnated such as with vaseline or silicone oil, may be used as a stationary phase, with relatively polar solvents as the moving phase.[2,11,12]

*Gas–Liquid Chromatography.* Retinol is rapidly converted int anhydroretinol under normal conditions of gas–liquid chromatography,[16] but it may be isolated with little destruction at 150 °C

by use of high flow rates on columns of 1% SE-30 on siliconized 60–80 mesh Gaschrome P that has been conditioned at 250 °C and treated with an antioxidant.[17]

**Methods of Assaying for Purity:**

*Chromatography.* The above methods of chromatography may be used to assay the purity of retinol.

*Ultraviolet Spectrum.* Retinol and its isomers each have a single light-absorption maximum. $E^{1\%}_{1cm}$ in ethanol; all-*trans*, 1832 (325 nm); 13-*cis*, 1686 (328 nm); 11-*cis*, 1220 (319 nm) or 945 (322 nm);[9] 9-*cis*, 1480 (323 nm); 9,13-di-*cis*, 1379 (324 nm); and 11,13-di-*cis*, 908 (311 nm). Several *cis*-isomers also have small absorption maxima ($E^{1\%}_{1cm}$ about 350) between 235 and 260 nm. Spectral properties of retinol derivatives have been collated.[8,10,13]

*Infrared Spectrum* The infrared spectrum of retinol has been reported.[8,9,12,18]

*Fluorescence Spectrum.* Light of wavelength 325 nm is absorbed maximally, and emitted at 470 nm.[12,18]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of retinol has been reported.[8]

*Melting Point.* The melting points of retinol and some of its isomers have been reported as follows:[6] all-*trans*, 62–64 °C (solvent free), 8 °C (methanol-solvated); 13-*cis*, 58–60 °C; 9-*cis*, 82–83 °C; and 9,13-di-*cis*, 58–59 °C.

**Other Properties:** Polarography of retinol[20] and the x-ray powder diagram of its crystals[2] have been reported.

**Quantitative Assays:** The quantity of retinol is usually determined by absorption of ultraviolet light.[8,10,18] Fluorescence of retinol may be measured.[21] Assays may be made by the Carr-Price reaction. Retinol forms transient, but intensely colored, complexes with antimony trichloride, trifluoroacetic acid,[22] or other Lewis acids. The $E^{1\%}_{1cm}$ value at 620 nm of this species is 5070. All isomers of retinol give the same complex. Retinol may also be dehydrated with acid to yield anhydroretinol, which is measured spectrophotometrically. $E^{1\%}_{1cm}$ values in ethanol are 2500 at 351 nm, 3650 at 371 nm, and 3180 at 392 nm.[23]

**Probable Impurities:** *cis*-Isomers and oxidation products of retinol are the most common impurities.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C). Peroxide-free ethyl ether and acid-free acetone or ethyl acetate are preferable to either ethanol or petroleum ether for storage. However, ethanol is suitable as a solvent for brief periods, for spectroscopic analysis.[24]

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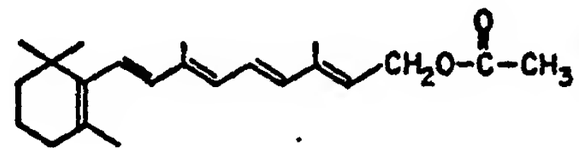
## Carot-41
## Retinyl Acetate
## (Vitamin $A_1$ Acetate)

**Formula:** $C_{22}H_{32}O_2$
**Formula Wt :** 328.50
**Calc. %:** C, 80.44; H, 9.82; O, 9.74




**Isomers:** The acetates of each of the six isomers of retinol have been synthesized.[1]

**Biopotency:** A U.S.P. unit or an International Unit (I.U.) of all-*trans*-retinyl acetate is 0.344 μg. Therefore, pure all-*trans*-retinyl acetate contains[2] $2.904 \times 10^6$ I.U./g.

**Sources:**

*Natural Sources.* Retinyl acetate is not found in natural materials.

*Chemical Synthesis.* Retinyl acetate is synthesized from retinol by treatment with acetic anhydride or acetyl chloride in pyridine, or from acetylated intermediates in the synthesis of retinol.[3]

**Methods of Purification:** Retinyl acetate may be separated from retinol by column chromatography. The compound may then be purified by crystallization from methanol. Purification methods were reviewed in 1960.[1]

**Methods of Assaying for Purity:**

*Column Chromatography.* Similar or identical adsorbents are used for the column chromatography of retinol and retinyl acetate.[4,5] On columns of water-deactivated alumina, retinyl acetate is eluted, after β-carotene, by hexane or 0.5% of acetone in hexane.

*Partition Chromatography.* Column-partition chromatography and reverse-phase paper chromatography have been used to separate retinyl acetate from retinol and other retinyl esters.[1]

*Gas–Liquid Chromatography.* Retinyl acetate may be assayed for purity by gas–liquid chromatography. However, retinyl acetate forms anhydroretinol during gas–liquid chromatography unless proper conditions are maintained.[8]

*Ultraviolet Spectrum.* The maximum for retinyl acetate differs from that of retinol in $E^{1\%}_{1cm}$ values only. The $E^{1\%}_{1cm}$ values reported for retinyl acetate in ethanol are: all-*trans*, 1560 (325–326 nm); 13-*cis*, 1430 (328 nm); 11-*cis*, 973 (320–321 nm); 9-*cis*, 1200 (323 nm); 9,13-di-*cis*, 1110 (324 nm); and 11,13-di-*cis*, 859 (310–311 nm).[1,8]

*Fluorescence Spectrum.* Retinyl acetate maximally absorbs light f 325 nm, and emits part of the energy at 470 nm.[1,6,7,8]

*Melting Point.* A value of 57–58 °C has been reported.

**Other Properties:** Infrared spectrum, nuclear magnetic resonance spectrum, and polarographic behavior are similar for retinol and retinyl acetate.

**Quantitative Assays:** Ultraviolet-light absorption and colorimetric analysis[1,6] are most commonly used in assays for quantity of retinyl acetate. Colorimetric assays may be made with the Carr-Price reagent (antimony trichloride), or with trifluoroacetic acid[9] or other Lewis acids. The $E^{1\%}_{1\,cm}$ value at 616 nm is 4420 for the colored species.

**Probable Impurities:** *cis*-Isomers, retinol, or oxidation products of retinyl acetate.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C). Retinyl acetate is more stable in peroxide-free ethyl ether, acid-free acetone, or acid-free ethyl acetate in the dark than it is in other solvents.[10]

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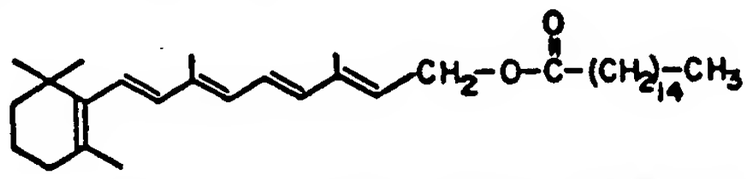
## Carot-42
## Retinyl Palmitate
## (Vitamin $A_1$ Palmitate)

**Formula:** $C_{36}H_{60}O_2$
**Formula Wt.:** 524.88
**Calc. %:** C, 82.38; H, 11.52; O, 6.10



**Isomers:** Six isomers of retinyl palmitate may be formed (see Retinol, Carot-40).

**Biopotency:** A U.S.P. unit or an International Unit of all-*trans*-retinyl palmitate is 0.55 μg. Thus, pure all-*trans*-retinyl palmitate contains $1.817 \times 10^6$ I.U./g.

**Sources:**

*Natural Sources.* Retinyl palmitate is the major ester of retinol found in liver, intestine, and retina of many vertebrates. Smaller amounts of stearate, oleate, and other esters are also present.[1–3]

*Chemical Synthesis.* Retinyl palmitate is synthesized by direct esterification of retinol with palmitoyl chloride in pyridine,[4] or by reaction with methyl palmitate in the presence of sodium ethoxide.[5]

**Methods of Purification:** Retinyl palmitate may be separated from retinol by column chromatography. It may then be crystallized from propylene oxide.[4]

**Methods of Assaying for Purity:**

*Column and Thin-Layer Chromatography.* Similar adsorbents are used for the column chromatography of retinol and retinyl palmitate.[6,7] Retinyl palmitate is eluted from columns of water-deactivated alumina with hexane or a very small percentage of acetone in hexane. Retinyl palmitate may also be chromatographed on a thin layer of silica gel G. Petroleum ether–isopropyl ether–acetic acid–water (180:20:2:5) or petroleum ether–acetonitrile–acetic acid–water (190:10:1:5)[8] are used to develop the chromatogram.

*Partition Chromatography.* Column-partition chromatography and reverse-phase paper chromatography have been used to separate retinyl palmitate from retinyl acetate and retinol.[7,8]

*Gas–Liquid Chromatography.* Retinyl palmitate does not emerge from gas–liquid columns at temperatures suitable for the chromatography of retinyl acetate and retinol.[9]

*Ultraviolet Spectrum.* Retinyl palmitate differs from retinol in its ultraviolet absorption spectrum in $E^{1\%}_{1\,cm}$ values only. In ethanol, all-*trans*-retinyl palmitate has an $E^{1\%}_{1\,cm}$ value of 1000 at 325 nm.

*Fluorescence Spectrum.* Light of 325 nm is absorbed maximally by retinyl palmitate. A portion of this energy is emitted[2] as light of 470 nm.

*Melting Point.* A value of 28–29 °C has been reported.

**Quantitative Assays:** The quantity of retinyl palmitate is normally determined by measurement of ultraviolet light absorbed,[7,8] or through measurement of light emitted by fluorescence.[2,8,10] Assays may also be made by measuring the absorbance of the colored complex formed with such Lewis acids as antimony trichloride and trifluoroacetic acid.[11] An $E^{1\%}_{1\,cm}$ value of 2760 is obtained at 616 nm.

**Probable Impurities:** Retinol, *cis*-isomers, and oxidation products.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (0 °C). Solutions of retinyl palmitate are reasonably stable in the dark at low temperatures in peroxide-free and acid-free organic solvents.[12]

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## Carot-43
## Spirilloxanthin
## 1,1′-Dimethoxy-3,4,3′,4′-tetrahydro-1,2,1′,2′-tetrahydro-ψ,ψ-carotene
## (Rhodoviolascin)

**Formula:** $C_{42}H_{60}O_2$
**Formula Wt.:** 596.95

Calc. %: C, 84.51; H, 10.12; O, 5.37; $OCH_3$, 10.40

**Sources:**

*Natural Sources.* Spirilloxanthin is found only in photosynthetic bacteria. *Rhodospirillum rubrum* (stationary-growth phase) is an excellent source of this compound.[1,2]

*Chemical Synthesis.* The total synthesis of spirilloxanthin has been reported.[3,4]

**Isolation Procedure:** The extraction, saponification, column chromatography, and crystallization of spirilloxanthin have been reported.[2,5–7]

**Methods of Purification:**

*Chromatography.* Column chromatography on either a calcium carbonate–calcium hydroxide mixture[6] or deactivated alumina[7] may be used to separate spirilloxanthin from other carotenoids.

*Crystallization.* Chloroform–petroleum ether,[6] acetone–petroleum ether,[7] benzene–petroleum ether,[7] or benzene[2,5] are used for crystallization. Spirilloxanthin is only slightly soluble in petroleum ether, moderately soluble in benzene, and readily soluble in acetone or carbon disulfide.

**Methods of Assaying for Purity:**

*Chromatography.* The purity of spirilloxanthin may be determined by chromatography on calcium hydroxide,[6] on circular filter paper having a suitable filler,[8,9] or by the thin-layer technique.[10]

*Solvent Partition.* The partition ratio[11] between petroleum ether and 95% methanol is 88:12.[7]

*Visible Spectrum.* Petroleum ether (b.p. 40–70 °C): 463, 493, and 528 nm. $E^{1\%}_{1cm}$ 2680 at 493 nm.[6] Spectral curve.[6,7] Acetone: 468, 498, and 534 nm. Chloroform: 479, 509, and 544 nm. Benzene: 480, 510, and 548 nm. Carbon disulfide: 495, 532, and 570 nm.

Iodine-isomerized spirilloxanthin shows "*cis*-peak" absorption at 367 and 385 nm in petroleum ether.[6]

*Infrared Spectrum.* The infrared absorption spectrum of spirilloxanthin in a potassium bromide pellet has been reported.[12]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of spirilloxanthin has been reported.[8,13]

*Mass Spectrum.* The mass spectrum of spirilloxanthin has been reported.[14]

*Melting Point.* Spirilloxanthin melts at 216–218 °C in an evacuated tube.[2,6]

**Probable Impurities:** Oxidation products, *cis*-isomers, and rhodopin or anhydrorhodovibrin when spirilloxanthin is isolated from natural sources.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and low temperature (−20 °C).

**References**


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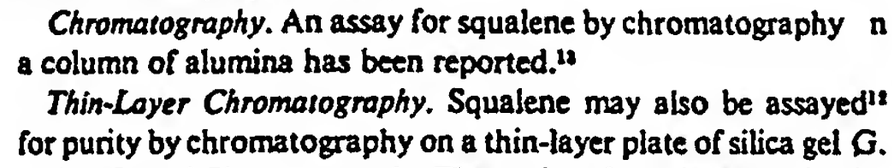
## Carot-44
## Squalene

Formula: $C_{30}H_{50}$
Formula Wt.: 410.74
Calc. %: C, 87.73; H, 12.27



**Sources:**

*Natural Sources.* Squalene is found in the largest amounts in fish-liver oils, particularly those of elasmobranchs,[1,2] Squalene is also found in plants.[3,4]

*Chemical Synthesis.* Several chemical syntheses of squalene have been reported.[5–9] In addition, it has been shown that natural squalene synthesized with tritium at C-12, has the *R* configuration.[10] It has also been shown that natural squalene is the all-*trans*-isomer.[11]

**Isolation Procedures:** Squalene is removed from biological materials by extraction with such solvents as acetone, methanol, or ethanol. The mixture of compounds in the extract is then subjected to saponification, and, subsequently, squalene and other nonsaponifiable compounds are transferred into petroleum ether.

**Methods of Purification:**

*Chromatography.* Squalene is purified by chromatography on a column of alumina, on a thin-layer plate, or by the gas–liquid technique.[12,13]

**Methods of Assaying for Purity:**

*Chromatography.* An assay for squalene by chromatography n a column of alumina has been reported.[13]

*Thin-Layer Chromatography.* Squalene may also be assayed[12] for purity by chromatography on a thin-layer plate of silica gel G.

*Gas–Liquid Chromatography.* The purity of squalene may be determined[13] by gas–liquid chromatography on a column of SE-30.

*Derivative Formation.* The hexabromides and hexachlorides of squalene have been prepared.[3] The thiourea clathrate of squalene has also been prepared,[4] and the biochemically important squalene 2,3-oxide has been synthesized.[14,15]

*Solvent Partition.* Squalene is insoluble in water, soluble in ether, petroleum ether, carbon tetrachloride, or acetone, and sparingly soluble in alcohol or glacial acetic acid.[16]

*Boiling Point.*[16] $b_{25}$, 285 °C; $b_4$, 250 °C; $b_{0.15}$, 203 °C.

*Density.* $d^{20}_4$ 0.8584 g/ml; $d^{20}_{20}$ 0.8538.

*Refractive Index.* $n^{20}_D$ 1.4965.

*Infrared Spectrum.* The infrared spectra of natural and synthetic squalene have been reported.[3]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of squalene has been reported.[17]

**Probable Impurities:** Oxidation products and *cis*-isomers.

**Conditions of Storage:** In darkness, at a low temperature (0 °C).

**References**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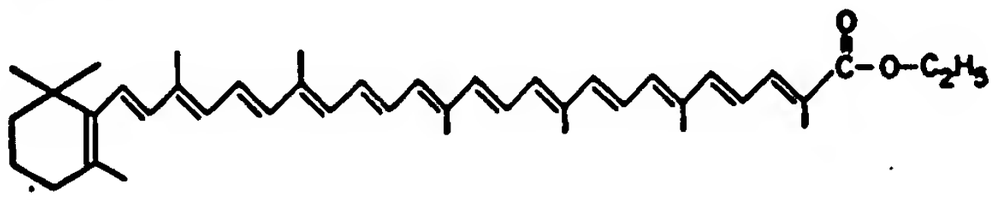
## Carot-45
## Torularhodin, Ethyl Ester
## Ethyl 3′,4′-Didehydro-β,ψ-caroten-16′-oate
## (β-$C_{40}$-Carotenoic Acid, Ethyl Ester)

**Formula:** $C_{42}H_{56}O_2$
**Formula Wt.:** 592.91
**Calc. %:** C, 85.08; H, 9.52; O, 5.40



**Sources:**

*Natural Sources.* Torularhodin has been isolated from microorganisms.[1–4]

*Chemical Synthesis.* The ethyl ester of torularhodin is prepared, in analogy to the methyl ester,[5] from β-apo-2′-carotenal ($C_{37}$) and [1-(ethoxycarbonyl)ethyl]triphenylphosphonium bromide.

**Methods of Purification:** This pigment is purified by chromatography on a column of deactivated alumina, and by crystallization from organic solvents (e.g., ethyl acetate).

**Methods of Assaying for Purity:**

*Chromatography.* This compound may be assayed for purity by chromatography on a column of deactivated alumina, or by chromatography on a thin-layer plate of secondary magnesium phosphate or silica gel G.[6] Ethyl acetate–dichloromethane (1:4), carbon disulfide, or benzene are used to develop the latter chromatograms.

*Visible Spectrum.* Torularhodin ethyl ester in hexane exhibits maxima at 475, 500, and 533 nm. $E^{1\%}_{1\,cm}$ (hexane) 2290, 3050, and 2430, respectively.

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of torularhodin ethyl ester has been reported.[7]

*Melting Point.* Torularhodin ethyl ester melts at 156–158 °C (uncorr.) in an evacuated tube.

**Probable Impurities:** *cis*-Isomers of the ethyl ester of torularhodin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and at a low temperature (−20 °C).

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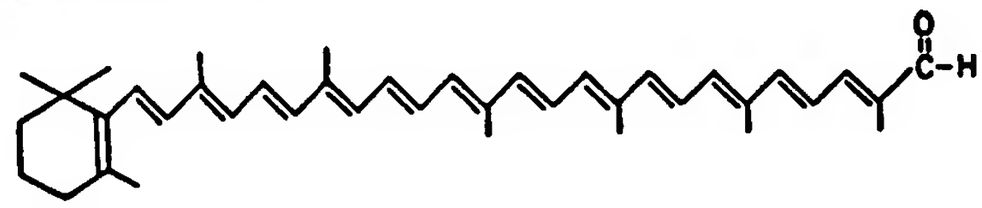
## Carot-46
## Torularhodinaldehyde
## 3′,4′-Didehydro-β,ψ-caroten-16′-al
## (3′,4′-Didehydro-17′-oxo-γ-carotene; β-$C_{40}$-Carotenal)

**Formula:** $C_{40}H_{52}O$
**Formula Wt.:** 548.86
**Calc. %:** C, 87.53; H, 9.55; O, 2.92



**Sources:**

*Natural Sources.* This compound has not been reported to be present in any natural sources other than *Rhodotorula* species,[1] where it is present in small amounts.

*Chemical Synthesis.* The synthesis of this compound from 15,15′-didehydro-β-apo-8′-carotenal by enol ether condensation has been reported.[2]

**Methods of Purification:** This compound may be purified by chromatography on columns of alumina, and by crystallization from organic solvents.[2]

**Methods of Assaying for Purity:**

*Chromatography.* The purity of this compound may be determined by chromatography on a column of alumina or on a thin layer of silica gel G (Merck). Ethyl ether–cyclohexane (1:4) is used to develop the latter chromatogram.[2]

*Visible Spectrum.* Petroleum ether (b.p. 80–105 °C): 509 and 540 nm (shoulder). Cyclohexane: 513 and 544 nm (shoulder). Benzene: 522 nm. The $E^{1\%}_{1\,cm}$ value at 508 nm is 2865 (petroleum ether, b.p. 80–105 °C).

*Melting Point.* A melting point of 166–168 °C has been reported.[2]

**Probable Impurities:** *cis*-Isomers of torularhodinaldehyde.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and at a low temperature (−20 °C).

**References**

1. R. Bonaly and J. P. Malenge, *Biochim. Biophys. Acta*, 164, 306 (1968).
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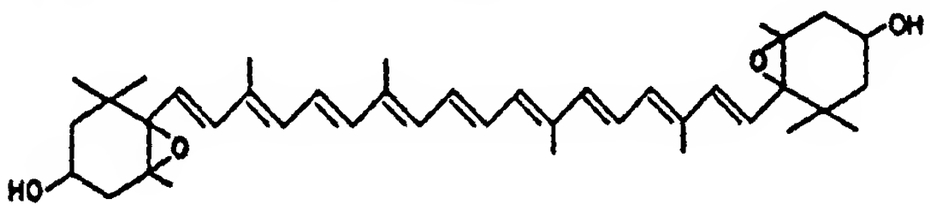
## Carot-47
## Violaxanthin
## (3*R*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol

Formula: $C_{40}H_{56}O_4$
Formula Wt.: 600.89
Calc. %: C, 79.96; H, 9.39; O, 10.65




Violaxanthin has been characterized as 5,6,5′,6′-diepoxy-zeaxanthin.[1] The stereochemistry of this compound is known to be 3*S*,3′*S*,[2,6] and the configuration of each 5,6-epoxide group is thought to be 5 *R*, 6 *S*.[6]

**Sources:**

*Natural Sources.* Violaxanthin is found in many flowers,[4] fruits,[4] green leaves,[5] and algae.[6,7] Crystalline violaxanthin has been isolated from *Viola tricolor*.[8]

*Chemical Synthesis.* Violaxanthin has allegedly been prepared, in low yield, by oxidation of zeaxanthin with monoperoxyphthalic acid.[3] However, the main product probably differs from natural violaxanthin in the configuration of the 5,6-epoxy groups.[2]

**Isolation Procedures:**[4] Yellow blossoms of *Viola tricolor* are dried, and then extracted with petroleum ether. The combined extracts are concentrated, and the material in the solution is saponified. Violaxanthin is then extracted into petroleum ether, and purified by crystallization or chromatography.

**Methods of Purification:**

*Solvent Partition.* A partial purification of violaxanthin can be achieved by solvent partition.[9]

*Chromatography.* Violaxanthin may be purified by chromatography on a column of magnesium oxide,[5,9] zinc carbonate,[10] or calcium carbonate.[4]

*Crystallization.* Violaxanthin can be crystallized from methanol or carbon disulfide.[11]

*Derivatives.* The di-(*p*-nitrobenzoate) and the dibenzoate of violaxanthin have been reported.[10]

**Methods of Assaying for Purity:**

*Chromatography.* The homogeneity of violaxanthin can be determined by chromatography on thin-layer plates[12] or on kieselguhr paper.[13]

*Solvent Partition.* An observed polarity of 2.49 and an $M_{50}$ of 66.2 have been reported[14] for this compound.

*Visible Spectrum.*[4] Carbon disulfide: 440, 470, and 501 nm. Chloroform: 424, 451.5, and 482 nm. Petroleum ether: 417.5, 443, and 472 nm. Ethanol: 417.5, 442.5, and 471.5 nm. $E^{1\%}_{1\,cm}$ approximately 2400 at 442.5 nm.[4,15] Spectral curves for violaxanthin have been published.[4,15]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum for violaxanthin has been reported.[2,16]

*Optical Rotatory Dispersion.* The optical rotatory dispersion curve for violaxanthin has been reported.[16]

*Mass Spectrum.* The mass spectrum of violaxanthin has been reported.[17]

*Melting Point.* Violaxanthin melts at 200 °C.[4]

*Optical Rotation.* An $[\alpha]^{20}_{Cd}$ of +35° (chloroform) has been reported for violaxanthin.[11]

*Derivatives.* Auroxanthin is formed on treatment of violaxanthin with dilute acid.[15]

*Color Reactions.* Violaxanthin gives a persistent blue color when an ethereal solution of this compound is shaken with 20% aqueous hydrochloric acid.[15]

**Probable Impurities:** Antheraxanthin, auroxanthin, and zeaxanthin.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and at a low temperature (−20 °C). Contact with acid vapors must be avoided.

**References**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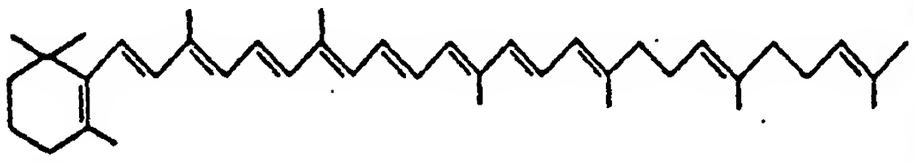
## Carot-48
## β-Zeacarotene
## 7′,8′-Dihydro-β,ψ-carotene
## (all-*trans*-7′,8′-Dihydro-γ-carotene)

Formula: $C_{40}H_{58}$
Formula Wt.: 538.90
Calc. %: C, 89.15; H, 10.85



**Sources:**

*Natural Sources.* β-Zeacarotene has been isolated from yellow corn grain,[1] yeast,[3] and fungi.[3]

*Chemical Synthesis.* The chemical synthesis of β-zeacarotene from farnesyl triphenylphosphonium bromide and 15,15′-didehydro-apo-12′-carotenal by a Wittig reaction has been reported.[4]

**Isolation Procedures:** β-Zeacarotene is extracted from biological materials with an organic solvent. The carotene is then transferred into petroleum ether, with or without prior saponification, and purified by chromatography.

**Methods of Purification:**

*Chromatography.* β-Zeacarotene is purified by chromatography on a column of magnesium oxide–Supercel,[1,2] alumina,[1,3] or calcium hydroxide–Celite.[1,3]

**Methods of Assaying for Purity:**

*Chromatography.* β-Zeacarotene may be assayed for purity by column chromatography as just noted. It may also be assayed for purity by chromatography on a kieselgel plate, or by thin-layer chromatography on alumina.[3]

*Visible Spectrum.* Petroleum ether: 406, 428, and 454 nm. $E^{1\%}_{1\,cm}$ 1660, 2520, and 2300, respectively.[4]

*Infrared Spectrum.* The infrared absorption spectrum of β-zeacarotene has been reported.[4]

*Nuclear Magnetic Resonance.* The nuclear magnetic resonance spectrum of β-zeacar tene has been reported.[5]

*Mass Spectrum.* The mass spectrum of β-zeacarotene has been published.[6]

*Melting Point.*[4] β-Zeacarotene melts at 96–97 °C.

**Probable Impurities:** Oxidation products, *cis*-isomers of β-zeacarotene, and, possibly, γ-carotene.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and at a low temperature (−20 °C).

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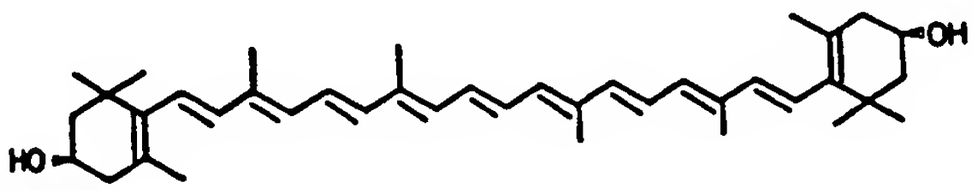
6. H. Budzikiewicz, H. Brzezinka, and B. Johannes, *Monatsh. Chem.*, 101, 579 (1970).


## Carot-49
## Zeaxanthin
## (3*R*,3′*R*)β,β-Carotene-3,3′-diol
## (β-Carotene-3,3′-diol)

**Formula:** $C_{40}H_{56}O_2$
**Formula Wt.:** 568.89
**Calc. %:** C, 84.45; H, 9.92; O, 5.62




**Sources:**

*Natural Sources.* Maize seeds,[1,2] calyx of *Physalis alkekengi* (as physalien, a zeaxanthin dipalmitate),[3–4] and in small proportions in other plant sources.[2,5,6] The absolute configuration of natural zeaxanthin from *Physalis alkekengi* and maize has been established[7,8] as 3*R*,3′*R*.

*Chemical Synthesis.* The total synthesis of zeaxanthin has been reported.[9,10]

**Isolation Procedures:** The isolation of zeaxanthin from natural sources involves extraction, saponification under mild conditions, extraction of the carotenediol with petroleum ether or ethyl ether, chromatography, and crystallization.[1–6,11]

**Methods of Purification:**

*Solvent Partition.* Zeaxanthin may be separated from carotenes by partition between petroleum ether and 95% methanol.[5,12]

*Chromatography.* Zeaxanthin may be purified by column chromatography on calcium carbonate, calcium hydroxide, zinc carbonate, magnesia, magnesium silicate, or deactivated alumina.[2,3,5,11]

*Crystallization.* Several solvent combinations may be used for crystallization. Two of these are carbon disulfide–ethyl ether–petroleum ether[4] and dichloromethane–methanol.[9] Ethanol or methanol may also be used. Zeaxanthin is almost insoluble in petroleum ether, slightly soluble in ethyl ether, and quite soluble in chloroform or carbon disulfide. One gram f the pigment dissolves in 1.5 liters of boiling methanol.[5]

*Derivatives.* Diesters[5] and diethers[13] f zeaxanthin have been reported.

**Methods of Assaying for Purity:**

*Chromatography.* The purity of zeaxanthin may be determined by chromatography on columns,[3] on circular paper having a suitable filler,[14] or on thin layers of adsorbent.[15]

*Solvent Partition.* The partition ratio between petroleum ether and 95% methanol is 11:89; and between petroleum ether and 85% methanol is 40:60.[13]

*Visible Spectrum.* Petroleum ether (b.p. 40–60 °C): 423, 452, and 480 nm. $E^{1\%}_{1\,cm}$ 2350 (452 nm) and 2050 (480 nm). Spectral curve.[5] Ethanol:[5] 423 (shoulder), 451, and 483 nm. Methanol:[5] 422 (shoulder), 450, and 481 nm. Chloroform:[5] 429 (shoulder), 462, and 495 nm. Carbon disulfide: 450 (shoulder), 482, and 517 nm. Iodine-isomerized zeaxanthin shows "*cis*-peak" light absorption at 336 nm (petroleum ether).[16]

*Infrared Spectrum.* The infrared spectra in chloroform and bromoform have been reported.[5]

*Nuclear Magnetic Resonance Spectrum.* The nuclear magnetic resonance spectrum of zeaxanthin has been reported.[17]

*Mass Spectrum.* The mass spectrum of zeaxanthin has been reported.[18]

*Optical Rotatory Dispersion.* The optical rotatory dispersion curve of zeaxanthin has been reported.[6]

*Melting Point.* Zeaxanthin melts at 205–206 °C in an evacuated tube.[9]

*Optical Rotation.* An $[\alpha]_{Cd}$ value of −40° to −42° (chloroform) has been reported.[19]

**Probable Impurities:** Oxidation products, *cis*-isomers, and possibly lutein.

**Conditions of Storage:** Darkness (brown vial), inert atmosphere (sealed ampoule), and at a low temperature (−20 °C).

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